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ABSTRACT

This four-volume student text is designed for use by Air Force personnel enrolled in a self-study extension course for medical laboratory technicians. Covered in the individual volumes are medical laboratory administration and clinical chemistry (career opportunities, general laboratory safety and materials, general medical laboratory administration, basic chemistry, solutions, photometry and spectrophotometry, automation, quality control in collection and handling of specimens); laboratory procedures in clinical chemistry (body metabolism; proteins, carbohydrates, and enzymes; liver and kidney function tests; gastric analysis; special chemistry tests; toxicology) and laboratory procedures in urinalysis (general considerations, renal functions, physical characteristics of urine, microscopic examination, and chemical examination). Each volume in the set contains a series of lessons, exercises at the end of each lesson, a bibliography, and answers to the exercises. Volume review exercises are also included. (MN)

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MEDICAL LABORATORY TECHNICIAN
(CHEMISTRY AND URINALYSIS)
(AFSC 92470)

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90411	MEDICAL LABORATORY TECHNICIAN (CHEMISTRY AND URINALYSIS) (AFSC 92470)	25 Jun 86

INSTRUCTIONS ***The following materials are needed to complete this course.*** ***Check this list*** *immediately upon receiving your course package, and if any materials are missing or incorrect* ***(numbers don't match), notify ECI*** *immediately. Use the ECI Form 17 for this purpose, and be sure to include your identification number, address, course and volume number, and VRE form designation (if a VRE is involved). Send all correspondence separately from your answer sheet.*[1]

ITEM	TYPE	DESIGNATION OR TITLE	INVENTORY CONTROL NUMBER	VRE ANSWER SHEET IDENTIFICATION
3	VOL	VOL 1, Introduction to Medical Laboratory Administration and to Clinical Chemistry	90411 01 8510	
4	VRE	VOLUME REVIEW EXERCISE (VOL 1)	90411 01 24	90411 01 24
5	VOL	VOL 2, Laboratory Procedures in Clinical Chemistry (Part I)	90411 02 7608	
6	VRE	VOLUME REVIEW EXERCISE (VOL 2)	90411 02 23	90411 02 23
7	VOL	VOL 3, Laboratory Procedures in Clinical Chemistry (Part II)	90411 03 7608	
8	VRE	VOLUME REVIEW EXERCISE (VOL 3)	90411 03 23	90411 03 23
9	VOL	VOL 4, Laboratory Procedures in Urinalysis	90411 04 7608	
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SPECIAL NOTE: DISREGARD THE AFSC 90470 PRINTED ON THE COVER OF THE TEXTS. THIS CDC IS FOR AIRMEN ON UPGRADE TRAINING TO AFSC 92470 ONLY.

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[1] *SEE REVERSE SIDE FOR ADDITIONAL INSTRUCTIONS.*

LIST OF CHANGES

COURSE NUMBER 90411	CAREER FIELDS, POLICIES, PROCEDURES AND EQUIPMENT CHANGE. ALSO, ERRORS OCCASIONALLY GET INTO PRINT. THE FOLLOWING ITEMS UPDATE AND CORRECT YOUR COURSE MATERIALS. PLEASE MAKE THE INDICATED CHANGES.
EFFECTIVE DATE OF SHIPPING LIST 25 Jun 86	

SPECIAL NOTE: THROUGHOUT THE TEXTS AND VOLUME REVIEW EXERCISES FOR THIS COURSE, CHANGE FIRST 3 DIGITS OF ALL AFSCs FROM 904 TO 924.

1. CHANGES FOR THE TEXT: VOLUME 1

a. Page 67, col 2, line 6 fr bot: Change "octet" to "completion."

b. Page 84, Exercise (052)-1.(10): Change "buffered" to "unbuffered."

c. Page 158, answer 011-1(7): Change "f" to "c."

d. Page 160, answer 029-3: Change "Take a few . . . results and report" to "Correct." Answer 033-7: Change "No" to "Yes" and delete "not" in both places.

e. Page 161, answer 042-10: Change "oxygen" to "hydrogen."

f. Page 163, answer 057-(1): Change "l" to "k."

g. Page 165, answer 074-8: After "as" add "those." Col 1, line 11 fr bot, answer 075-6: Change "serum" to "the cells."

2. CHANGES FOR THE TEXT: VOLUME 2

a. Page 52, col 2, last line: Change "LDH" to "LAP."

b. Page 53, col 1, lines 1 and 3: Change "LDH" to "LAP."

3. CHANGE FOR THE TEXT: VOLUME 3

Page 56, col 1, line 12: Change "facilities" to "fatalities." Col 2, line 12: Change "423" to "433."

4. CHANGES FOR THE VOLUME REVIEW EXERCISE: VOLUME 1

a. Page 6, question 34: In the stem of the question, change "service." to "service; however, this was not expected to cover the manning shortage in the blood bank."

b. Question 10 is no longer scored and need not be answered.

5. CHANGES FOR THE VOLUME REVIEW EXERCISE: VOLUME 2

a. Page 3, question 15, choice _a_: Change "incerase" to "increase." Choice _b_: Change the second "decrease" to "increase."

b. Page 5, question 31: In the stem of the question, delete ", serum bilirubin is high and."

c. Page 6, question 38: In the stem of the question, change "Frog" to "Grof."

d. Page 9, question 72, choice _a_: Change "phenlphethalein" to "phenolphthalein." Choice _c_: Change "methly" to "methyl."

e. Page 11, question 81: In the stem of the question, change "catalyze" to "catalyzes."

LIST OF CHANGES

COURSE NUMBER 90411	CAREER FIELDS, POLICIES, PROCEDURES AND EQUIPMENT CHANGE. ALSO, ERRORS OCCASIONALLY GET INTO PRINT. THE FOLLOWING ITEMS UPDATE AND CORRECT YOUR COURSE MATERIALS. PLEASE MAKE THE INDICATED CHANGES.
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5. CHANGES FOR THE VOLUME REVIEW EXERCISE: VOLUME 2 (Continued)

f. Page 13, question 105, choices a and c: Change "exalacetate" to "oxalacetate."

g. The following questions are no longer scored and need not be answered: 47 and 81.

6. CHANGES FOR THE VOLUME REVIEW EXERCISE: VOLUME 3

a. Page 2, question 5, choice a: Change "ureas" to "urea."

b. Page 3, question 11: In the stem of the question, change "text" to "test." Question 14: In the stem of the question, change "insufficiently" to "insufficiency."

c. Page 7, question 44, choice c: Change "nitrose" to "nitroso." Question 59: In the stem of the question, change "materia" to "material."

d. Page 9, question 64, choice d: Change "dodium hydrosulfide." to "sodium hydrosulfite." Question 68: In the stem of the question, change "gives a positive test" to "will cause the immediate appearance of a slight turbidity."

e. The following questions are no longer scored and need not be answered: 49 and 65.

7. CHANGES FOR THE VOLUME REVIEW EXERCISE: VOLUME 4

a. Page 3, question 15: In the stem of the question, change "pressure" to "presence."

b. Page 5, question 31: In the stem of the question, after "solution" add a comma.

c. Page 6, question 40: In the stem of the question, remove underline from "describing" and under line "transudation."

d. Page 10, question 80, choice c: Change "Abermayer" to "Obermayer." Question 84, choice d: "orthotolodine" to "orthotolidine."

e. Page 11, question 88: Change "(115)" to "(638)."

f. Question 72 is no longer scored and need not be answered.

90411 01 8510

CDC 90411

MEDICAL LABORATORY TECHNICIAN—CLINICAL CHEMISTRY AND URINALYSIS

(AFSC 92470)

Volume 1

Introduction to Medical Laboratory Administration and to Clinical Chemistry

QUALITY
SERVICE
PROFESSIONALISM

Extension Course Institute
Air University

Prepared by
CMSgt Joselyn H. Thompson
(AUTOVON: 736–2809)

Reviewed by
Elmore C. Hall

Edited by
Joyce Wagner Anderson

SCHOOL OF HEALTH CARE SCIENCES, USAF (ATC)
SHEPPARD AIR FORCE BASE, TEXAS 76311–5465

EXTENSION COURSE INSTITUTE (AU)
GUNTER AIR FORCE STATION, ALABAMA 36118–5643

Preface

THE AIR FORCE laboratory technician should possess and demonstrate the technical ability to effectively deliver quality laboratory service. Quality laboratory service is essential to the accurate diagnosis and treatment of patients. Laboratory technicians, today and in the future, should use good supervisory and administrative skills for efficient management of the medical laboratory.

This volume provides you with a background of basic information so that together with your on-the-job training and experience, you should easily make the transition from the 5-level specialist to the 7-level technician.

This CDC 90411 is the first of three career development courses for the 92470 specialty. Chapter 1 of this volume introduces the mission and capabilities of Air Force clinical laboratories and guidelines in career progression. In chapter 2, you will review current principles of laboratory materiel and general laboratory safety. Chapter 3 reviews general laboratory administration, emphasizing laboratory ethics and interpersonal relations, laboratory reports, and accreditation program. Chapters 4 and 5 cover a review of basic chemistry and the nature and concentration of laboratory solutions. Chapter 6 is about basic principles of photometry and spectrophotometry. Chapter 7 reviews principles, techniques, and instruments used in automation. Chapter 8 concludes with further principles and techniques for collection and handling laboratory specimens and a review of quality control in the clinical laboratory.

For your convenience in the course and future reference, the Appendixes contain the duties and responsibilities of your specialty, a Periodic Chart, and a summary of general laboratory formulae.

Code numbers appearing on figures are for preparing agency identification only.

The inclusion of names of any specific commercial product, commodity, or service in this publication is for information purposes only and does not imply indorsement by the Air Force

NOTE: If you know this course contains erroneous or outdated information or does not provide the knowledge that the current Specialty Training Standard (STS) requires you to have for upgrade training, contact your unit OJT advisor and fill out AF Form 1284, Training Quality Report. If you need *immediate* clarification of information in these study materials, call the author between 0700 and 1600 (CT), Monday through Friday.

Consult you education officer, training officer, or NCO if you have questions on course enrollment or administration, Your Key to a Successful Course, and irregularities (possible scoring errors, printing errors, etc.) on the volume review exercises and course examination. Send questions these people can't answer to ECI, Gunter AFS AL 36118-5643, on ECI Form 17, Student Request for Assistance.

This volume is valued at 45 hours (15 points).

Material in this volume is technically accurate, adequate, and current as of April 1985.

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NOTE: This course teaches through numbered lesson segments, each containing a behavioral objective, text, and exercises. The objective sets your learning goal. The text gives you the information you need to reach that goal, and the exercises let you check your achievement. When you complete each segment, see whether your answers match those in the back of the volume. If your response to an exercise is incorrect, review the objective and its text.

CHAPTER 1

Introduction to the Medical Laboratory Career Field

TO BE SUCCESSFUL, you, the laboratory technician, must not only possess the expertise in the medical, scientific, and technical areas, but must also be able to work effectively as managers and supervisors. If your laboratory must provide the highest quality of service in accomplishing the clinical laboratory mission, you must prepare to accept the challenge of performing a significant number of administrative laboratory tasks. These may range from the monitoring of training and supervision of laboratory personnel or the requisitioning and maintaining of adequate quantities of laboratory supplies and equipment to preparing complicated reports that are submitted to higher headquarters. However, most of our administrative procedures involve preparing and maintaining accurate reports and records.

The chapter discusses, in its first section, the organization of the USAF Medical Laboratory Service. Then, to establish your role in this organization, your medical laboratory career field is explained. Finally, the last section emphasizes your responsibilities and duties as a laboratory supervisor and indicates the important role you share in the efficient performance of medical laboratory administration.

1-1. The USAF Medical Laboratory Service

The medical laboratory has evolved from a basic hospital service to an enormous business entity. The traditional role of producing laboratory results to aid in the diagnosis of diseases and to follow the progress of therapy has been significantly increased. Today, the laboratory has become a very essential and visible part of the services provided by the hospital. You, as a medical laboratory technician, perform a vital role in the accomplishment of the clinical laboratory mission.

001. State the mission of the USAF Medical Laboratory Service, list the factors that determine the services that a laboratory provides, and identify the classes of laboratories that act as consultant, reference, and support laboratories.

Mission of the Clinical Laboratory. The mission of the USAF Medical Laboratory Service is to provide timely and reliable laboratory support of value in the diagnosis, treatment, and prevention of diseases affecting the health and welfare of U.S. Air Force personnel and their dependents. In all instances, the analyses you perform are indispensable if the Medical Service is to properly minister to the health needs of those it serves.

The Medical Laboratory Service is organized as a part of the USAF Medical Service and is under the supervision of the Surgeon General, USAF. The senior laboratory officer is a colonel and is an associate chief of the Biomedical Science Corps. The amount and variety of service a medical laboratory provides depends upon the medical facility it supports. Since USAF medical facilities vary from the large treatment center hospital to the smallest medical unit, so does the size of medical laboratories which support them. The size of the medical facility alone, however, does not always dictate the nature and extent of laboratory services provided. Some laboratories have a fully developed laboratory service, while others ship many specimens to other laboratories for routine or special analyses. The scope of treatment provided by the medical facility and the number, initiative, and/or training of laboratory personnel assigned are also factors that determine the workload of a given medical laboratory.

AFR 160-32, *Clinical Laboratory Classification and Capabilities*, states that Air Force medical laboratories are categorized into four classes on the basis of personnel assigned and anticipated workload.

Classification of Laboratories. The four classes are designated on the basis of capabilities to perform the various laboratory procedures.

Class A. Class A laboratories are located at area medical centers in the States or at the larger overseas medical treatment facilities where multiple medical specialties are represented. They are equipped and staffed to give complete clinical laboratory support to the specialized treatment facility. They also serve as consultant, reference, and support laboratories to other Air Force medical facilities within specified geographical regions.

Class B. These laboratories generally are located at regional hospitals. At least one pathologist and one biomedical laboratory officer are authorized. In addition to conducting tests and examination for their medical facility, they may conduct certain tests for nearby installations when the respective hospital commanders can make satisfactory arrangements. Tests or examinations that Class B laboratories are not able to complete are forwarded to Class A laboratories or the Epidemiology Division USAF SAM or to Army or Navy laboratories in accordance with AFR 161-40, *Joint Utilization of Certain Armed Forces Medical Laboratory Facilities*.

Class C. Class C laboratories have no pathologist but at least one biomedical laboratory officer authorized. They ordinarily conduct laboratory tests and examinations only for their installation. Tests that they cannot perform are likewise forwarded to other laboratories as indicated for Class B laboratories.

Class D. These laboratories are located at small medical facilities with neither a pathologist nor a biomedical laboratory officer authorized. They are equipped and staffed to perform a limited number of laboratory tests and examinations depending on the scope of medical care given. Essentially, they provide the necessary laboratory support for physical examinations and limited laboratory services required in support of a USAF clinic. They may offer fewer tests than those listed within the range of their class capability. Tests that they cannot perform are forwarded to other laboratories as those for Class B and C laboratories.

When expediency and time economy is of greatest concern, tests and examinations are referred to other commercial laboratories "downtown," or community hospital laboratories or to state public health laboratories. This service is occasionally provided upon the request of the staff physicians in order to enhance the quality and effectiveness of treatment.

Exercises (001):

1. What is the mission of the USAF Medical Laboratory Service?

2. What factors determine the amount and variety of service provided by a medical laboratory?

3. Tests or examinations that are not done by Class B, C, and D laboratories may be sent to which other military laboratories?

4. To which other nonmilitary facilities are tests and examinations referred?

5. Match the following classes of laboratories in column B with the type of services, treatment, support, and staffing in column A. Each column B item may be used once or more than once.

Column A	*Column B*
____(1) At least one biomedical laboratory officer assigned with no patholologist.	a. Class A.
____(2) These laboratories are equipped and staffed to support limited medical care such as physical exams.	b. Class B.
____(3) These laboratories are generally located at regional hospitals and may conduct tests for nearby installations.	c. Class C.
____(4) At least one pathologist and one biomedical laboratory officer are authorized.	d. Class D.
____(5) Located at area medical centers and serve as consultant, reference, and support laboratories to other Air Force medical facilities within specified geographical regions.	
____(6) They are equipped and staffed to give complete clinical laboratory support.	
____(7) Are located at small medical facilities with neither a pathologist nor a biomedical laboratory officer assigned.	

1-2. The USAF Medical Laboratory Career Field

You have one of the most responsible and demanding Air Force specialities. As a member of the Medical Laboratory Career Field, you may perform the duties of an apprentice specialist, a specialist, a technician, or a superintendent. A significant number of the tests that you do will directly affect the outcome of the treatment administered to a patient. The patient's life and well-being may depend upon your acquired knowledge, skill, and integrity.

002. Name the directive in which you can find an explanation of your Air Force Speciality Code and cite the significance of each digit in your AFSC.

The U.S. Air Force Specialty Code. A graphic explanation of the significance of the digits of your Air Force Specialty Code is found in AFR 35–1, *Military Personnel Classification Policy*. These terms will help you to understand the line progression and qualification which comes as a result of promotion in a given specialty. The code is composed of five digits. Here is the way your AFSC for the Medical Laboratory Technician, 92470, is identified.

a. The first two digits of your AFSC indicate the career field. Thus, the 92 stands for a component of the Medical Career Field.

b. The third digit identifies a subdivision of the career field. The 4 represents the Medical Laboratory Career Field subdivision within the medical field.

c. The fourth digit identifies the skill level. The level may be shown as 1, 3, 5, 7, or 9. The progression from 1 to 9 represents the way you may advance in the AFSC as your skill, knowledge, and scope of responsibility increase. The 1 represents the helper level; the 3, the semiskilled level, the 5, the skilled level; the 7, the advanced level; and the 9, the superintendent level.

d. The fifth digit is used to show a further subdivision within the career field. The 0 denotes the clinical laboratory field, and 1 is for histopathology technicians. The cytotechnology technician is identified as 92570.

Exercises (002):

1. In what Air Force regulation would you find a description and explanation of terms pertaining to the Air Force Specialty Code?

2. What does the code 925 represent?

3. Match each digit of your AFSC (92470) with the correct meaning in column B.

Column A	*Column B*
______(1) 92.	a. Skill level.
______(2) 4.	b. Subdivision of career field.
______(3) 7.	c. Medical Career Field.
______(4) 0.	d. Further subdivision of career field.

003. Cite the basic educational requirements for an airman to enter the Medical Laboratory Career Field and the requirement that must be met by individuals who were trained at civilian medical laboratory schools.

Basic Educational Requirements. As you may recall, certain experience and educational qualifications have a bearing on selection of an airman for a specific career field. High school courses in chemistry and algebra are mandatory and courses in biology, zoology, and other basic sciences desirable for one who is to train for AFSC 92430. Experience in performing medical laboratory procedures in urinalysis, hematology, bacteriology, serology, and clinical chemistry is mandatory for entry into training for AFSC 92450. This requirement is satisfied either by completing the Medical Laboratory Specialist Course 3ABR92430 or by having had equivalent training in civilian schools. Individuals who were trained in civilian schools, however, are sent directly to the School of Health Care Sciences at Sheppard AFB. They are evaluated by a selected corp of qualified instructors and supervisors of the Apprentice Medical Laboratory Specialist Course. If selected, they are proficiency advanced through course 3ABR92430. We shall describe the training requirements in more detail later in this chapter.

Exercises (003):

1. Compare mandatory with desirable educational requirements for selection of airmen for entry into the Medical Laboratory Career Field.

2. To be allowed to enter into 92450 training, what requirement must be met by individuals who were trained in civilian schools?

004. Cite progression policies in the 924X0 career field in terms of requirements, Air Force training resources, tests, and regulation in which information about career progression may be found.

Career Progression. For advancement to AFSC 92450, you must complete the Medical Laboratory Specialist Phase II Course (5AZO92450) offered at selected USAF hospitals. In order to advance to AFSC 92470, Medical Laboratory Technician, qualification as a medical laboratory specialist (AFSC 92450) is mandatory. In addition, experience in performing and supervising medical laboratory activities, such as work normally performed at Air Force clinics or hospitals, technical supervisory experience in clinical chemistry, bacteriology, toxicology, or urinalysis at Class A, B, and C or similar laboratories, or a combination of the foregoing experience is mandatory. Professional Military Education (PME) Phase II, USAF Supervisor's Course, or PME Phase III, Command NCO Leadership School, is mandatory for advancement from the 5 to the 7 level. In addition, the Career Development Courses 90411, 90412, and 90413 must be completed before you can advance from the 5 to the 7 level. Another requirement is at least 12 months of OJT. The successful completion of your Career Development Courses will help to increase your technical knowledge of all phases of the clinical laboratory. Continuous review will help to improve your score on the SKT and enhance your chances for promotion. You must also be in the appropriate pay grade and meet all eligibility requirements for promotion to the particular skill level.

The medical laboratory career ladder is shown in figure 1-1. The illustration is essentially based on AFR 35-1,

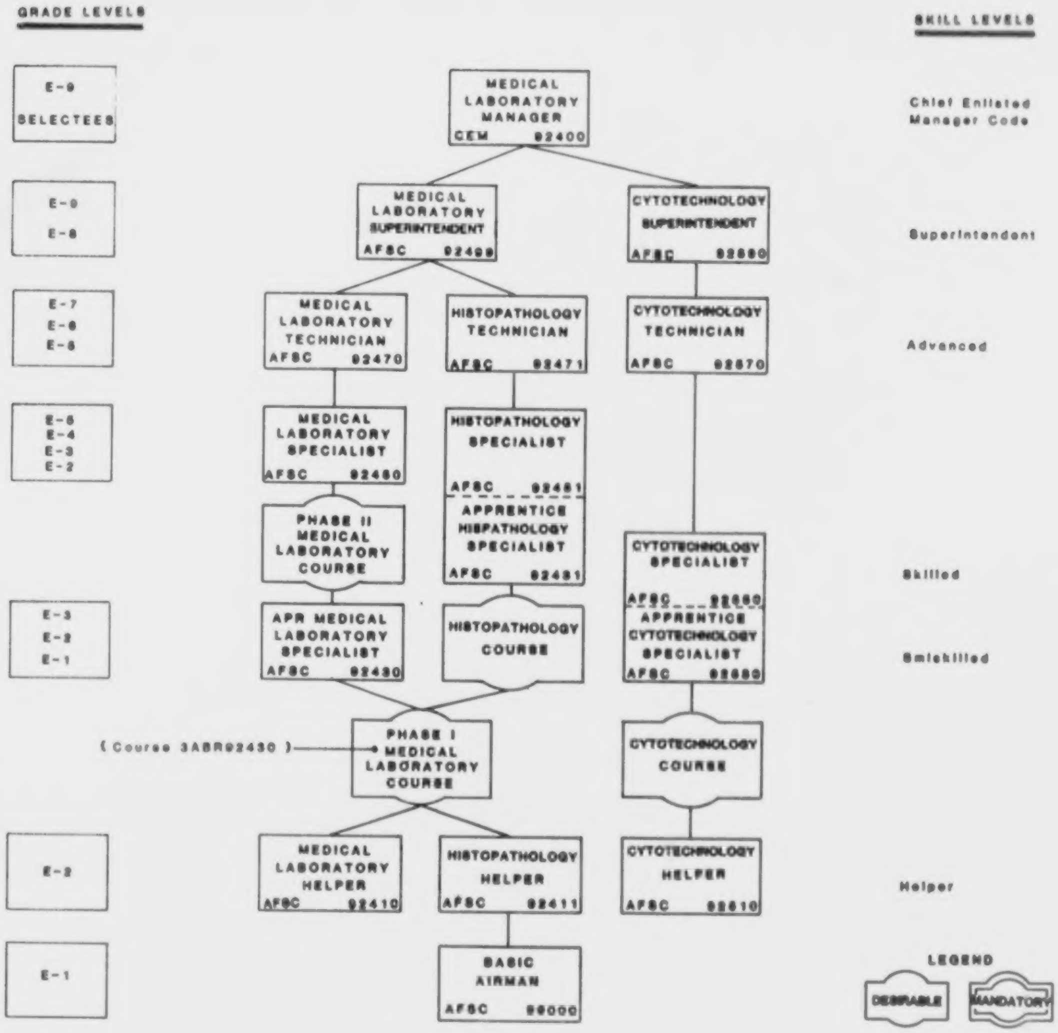

GRADE LEVELS
SKILL LEVELS
E-9
SELECTEES
MEDICAL LABORATORY MANAGER
CEM 92400
Chief Enlisted Manager Code
E-9
E-8
MEDICAL LABORATORY SUPERINTENDENT
AFSC 92490
CYTOTECHNOLOGY SUPERINTENDENT
AFSC 92590
Superintendent
E-7
E-6
E-5
MEDICAL LABORATORY TECHNICIAN
AFSC 92470
HISTOPATHOLOGY TECHNICIAN
AFSC 92471
CYTOTECHNOLOGY TECHNICIAN
AFSC 92570
Advanced
E-5
E-4
E-3
E-2
MEDICAL LABORATORY SPECIALIST
AFSC 92450
HISTOPATHOLOGY SPECIALIST
AFSC 92451
APPRENTICE HISPATHOLOGY SPECIALIST
AFSC 92431
PHASE II MEDICAL LABORATORY COURSE
CYTOTECHNOLOGY SPECIALIST
AFSC 92550
APPRENTICE CYTOTECHNOLOGY SPECIALIST
AFSC 92530
Skilled
E-3
E-2
E-1
APR MEDICAL LABORATORY SPECIALIST
AFSC 92430
HISTOPATHOLOGY COURSE
Semiskilled
(Course 3ABR92430)
PHASE I MEDICAL LABORATORY COURSE
CYTOTECHNOLOGY COURSE
E-2
MEDICAL LABORATORY HELPER
AFSC 92410
HISTOPATHOLOGY HELPER
AFSC 92411
CYTOTECHNOLOGY HELPER
AFSC 92510
Helper
E-1
BASIC AIRMAN
AFSC 99000
LEGEND
DESIRABLE
MANDATORY

Figure 1-1. Medical Laboratory Career Chart.

Military Personnel Classification Policy (Officers, Warrant Officers, Airmen), and AFR 39-1, *Airmen Classification Regulation*. Individual specialty levels in the airman career field are defined in AFR 39-1. A specialty description consists of four sections: (1) specialty summary, (2) duties and responsibilities, (3) specialty qualifications, and (4) specialty shredouts (when applicable). Please note Appendixes A, B, C, D, and E for specialty descriptions associated with the 924X0 career field.

The airman classification structure is designed to afford an opportunity for you to progress in your field through jobs of increasing responsibility and rank. Note in figure 1-1 that the Course 3ABR92430, Apprentice Medical Laboratory Specialist Phase I, is mandatory in order to progress to the 5 level.

Terms. When discussing or reading about advancement procedures in your career area, you may encounter certain terms. Let's consider these terms now.

Apprentice Knowledge Test (AKT). This is a comprehensive, multiple-choice, written test designed to measure technical knowledge in a semiskilled Air Force specialty. There is no AKT required for entry into the 924X0 AFSC.

Specialty Knowledge Test (SKT). The SKT is a comprehensive, multiple-choice written test which measures technical knowledge in the skilled or advanced AFSCs. All airmen eligible for promotion to E–5 through E–7 are administered an SKT annually in the 924X0 AFSC.

On-the-job training (OJT). This is a training program which qualifies an individual (through supervised instruction) in the performance of his or her duties in a given Air Force specialty.

Classification board. A classification board is a group of specially qualified officers, warrant officers, and noncommissioned officers convened by the appointing authority for the purpose of recommending personnel classification actions.

USAF supervisory examinations. USAFSE is a multiple-choice written test which measures the supervisory knowledge required in superintendent specialties.

Basic course. A basic course is a formal course which trains you toward the skilled level of a specialty in the career field ladder. It usually results in an award of the AFSC at the semiskilled level.

Lateral course. A lateral course is a formal course that trains you toward an AFSC in a career field ladder which required (before entry) initial qualification at the semiskilled or higher level in the AFSC or in a related career field ladder.

Bypassed specialist. A bypassed specialist is an airman who has been awarded the semiskilled level of an AFSC based on prior civilian or military experience or schooling.

Exercises (004):

1. In order to advance to the 7-level AFSC 92470, what requirements must be satisfied?
2. To obtain information about your specialty description, for example AFSC 92470, to what regulation would you refer?
3. Describe the Specialty Description for the AFSC 92470.
4. What AKT is required for entry into the 924X0 AFSC?
5. How does the AKT differ from the SKT?
6. What formal course trains you toward the skilled level of a specialty in a career field?
7. What formal course trains you toward an AFSC in a career field ladder which required (before entry) initial qualification at the semiskilled level or higher?
8. What is a bypassed specialist?

005. Cite prerequisites for proficiency advancement through course 3ABR92430 and the requirements for progression from AFSC 92410 to AFSC 92450 and to the rank of E–4.

Bypassed Specialist. When airman are qualified by virtue of prior civilian or military occupational experience or training, as determined by the career interview at Lackland AFB, they are later assigned to the School of Health Care Sciences at Sheppard AFB, Texas. They are evaluated by a selected group of qualified members of the instructor staff. Their education record is evaluated, and they are given ample time to prepare to take a series of written block examinations administered by the instructor staff. If the airmen obtain qualifying scores, they are also administered a practical examination. Passing results obtained from both the administered block tests and the practical examination qualifies the individual for entry into the Medical Laboratory Specialist Course ABR92430. All airmen meeting such requirements are proficiency advanced through Course 3ABR92430. They are awarded the AFSC 92430 prior to reassignment to Phase II training.

AFSC 92430. Airmen completing basic training who are scheduled for training in the medical laboratory career field are assigned to Sheppard AFB to attend Course 3ABR92430. An airman basic (E–1) may be promoted to E–2 on completion of 6 months time in grade or the day after graduation from basic military training (BMT), if enlistment contract guarantees E–2 after completion of BMT. In addition, an airman (E–2) may be promoted to airman first class (E–3) on the day after graduation from basic military training (BMT), provided the airman is serving on an initial 6-year enlistment.

Course 3ABR92430 (Phase I) consists of 17 weeks of instructions with emphasis on the theoretical aspects of medical technology. The curriculum includes subjects that enable the students to meet the knowledge and performance level required by the 3-skill level proficiency codes of the Specialty Training Standard (STS) for the 924X0 AFSC.

TABLE 1-1

ELIGIBILITY REQUIREMENTS FOR PROMOTION

FOR PROMOTION TO GRADE OF	MINIMUM MONTHS IN GRADE REQUIRED	MINIMUM TIME IN SERVICE REQUIRED	SKILL LEVEL
CMSgt (E-9)	24	14 Yrs	9
SMSgt (E-8)	24	11 Yrs	7 or 9
MSgt (E-7)	24	8 Yrs	7
TSgt (E-6)	18	5 Yrs	7
SSgt (E-5)	6	3 Yrs	5
SrA (E-4)	8	1 Yr	5
A1C (E-3)	6	-	-
Amn (E-2)	6	-	-

After successful completion of this course, students are awarded AFSC 92430.

AFSC 92450. All graduating students from Phase I are assigned advanced training at one of the Phase II Air Force hospitals for 36 weeks of practical clinical laboratory training in Course 5AZO92450. Students receive classroom as well as clinical training, which together satisfy the requirements of the knowledge and task levels as indicated for the 5 level in the STS. The commander recommends the promotion of the airman to airman first class on the completion of 6 months' time in grade.

On the effective date an airman may be promoted to E-4 after completing at least 8 months time in grade (TIG) as an E-3 and 12 months total Federal military service. Since promotions to grade E-4 are dependent on vacancies, the actual promotion to E-4 normally occurs around the 22nd month TIG.

Airmen first class may compete for early advancement to E-4 under the Below-The-Zone (BTZ) promotions. Unit commanders may nominate up to 10 percent of their eligibles to compete for BTZ promotion. Except of cross-trainees, the highest rank permitted for a 5 level is staff sergeant. Note table 1-1 for eligibility requirements for promotion.

Exercises (005):

1. What basic experience qualifies an airman for entry into Course 3ABR92430 by proficiency advancement?

2. What two types of examinations must be passed before entry into Course 3ABR92430 as a bypassed specialist?

3. What AFSC and duty title is awarded after successful completion of Course 3ABR92430?

4. What AFSC and duty title is awarded after successful completion of Course 3AZO92450?

5. How much time in grade must an airman complete before recommendation for promotion and officially by whom?

6. Since promotions to grade E-4 are dependent on vacancies, the actual promotion to E-4 normally occurs around what month of time in grade?

7. Under what promotion system can the airman first class compete for early advancement?

8. What percent of eligibles are nominated by commanders to compete for the BTZ promotion to E-4?

9. Except for cross-trainees, what is the highest rank permitted for a 5 level?

006. Cite time and course requirements for progression to AFSC 92470.

AFSC 92470. The next step up the medical laboratory career ladder is training to the "advanced" skill level. Training to this skill level is a bit more involved, as you are now discovering, than the others, so let's begin by looking at the requirements necessary to begin training.

Initial requirements. Before training is initiated to the 7 level, possession of AFSC 92450 is an obvious requirement. Another requirement is that the rank of staff sergeant must be achieved—that is, it must be "on the sleeve" or "in the bag" as opposed to possession of a line number. Career airmen must begin training when promoted to staff sergeant. First term staff sergeants have an option, and if they reject training they must sign a statement to the effect.

Training requirements. There are several requirements that you must meet before you are awarded AFSC 92470. One is successful completion of this CDC, 90411, plus 90412, and 90413. You must complete the 11 volumes within 1 year from the date of enrollment, or your enrollment is canceled. You may request your OJT supervisor to ask ECI for an extension if, due to unusual circumstances, you are unable to complete the three courses in 1 year. The request must be made to ECI before the end of the 12th month.

Another requirement is at least 12 months of OJT. This training, along with that of the CDC, enables you to meet the 7-level coding requirements of the STS.

As emphasized earlier under career progression, PME Phase II, USAF Supervisor's Course, or PME Phase III, Command NCO Leadership School, must be completed before you obtain your 7 level.

The last major requirement is that your supervisor must recommend you for upgrading to the 7 skill level.

Once you begin your 7-level training, you have 2 years in which to meet all the requirements. At the end of 2 years, if you have not completed the requirements, you must meet a classification board. The board decides whether sufficient training has been given to an airman who is in 7-level training and who has failed to qualify for the 7 level within the specified time period allowed. If it is determined that an airman has not, in fact, been given adequate training during the normal training time, the board will recommend an extension of time to complete the training. If an airman has failed to progress in training and the board finds that training has been adequate, the board will recommend an appropriate classification and training action. They may recommend any of the following actions:

a. Withdrawal of AFSC and return to duty in an awarded AFSC.

b. Withdrawal of AFSC and retrain into another AFSC more compatible with capabilities.

c. Remove from training.

Except for cross-trainees, the highest rank permitted with AFSC 92470 is master sergeant.

Exercises (006):

1. When may a medical laboratory specialist begin training for his or her 7 level?

2. How long do you normally have to complete this series of CDCs?

3. How may you receive an extension to complete this CDC?

4. What minimum time in training status is necessary before you may be awarded AFSC 92470?

5. Name one of the two PME courses that must be completed before the 7 level is awarded.

6. What action will the classification board recommend if an airman has not been given adequate training during the normal training time?

7. Except for cross-trainees, what is the highest rank permitted with AFSC 92470?

007. Cite requirements for progression to AFSC 92499 and to the rank of chief master sergeant.

AFSC 92499, Medical Laboratory Superintendent. Your 7-level AFSC will support your promotions to technical sergeant and master sergeant. If you are promoted to E-8 or selected for promotion to E-8, then you are qualified for award of your 9 level. You may also be awarded your 9 level if you are in pay grade E-7 for a minimum of 12 months; possess your 7 level; are recommended by your supervisor, and have completed the USAF Senior NCO Academy Associate Program.

Selections for promotion to senior master sergeant and chief master sergeant are made by promotion boards meeting annually at Headquarters Air Force Military Personnel Center, Randolph AFB, Texas. The evaluation board panel consists of two colonels and one chief master sergeant. Generally, the same panel considers the records of all airmen competing for promotion in a single AFSC.

Before the actual selection, a trial run is conducted to familiarize members with the evaluation process, content of records, and scoring methods, and to establish a board standard for evaluating records. This standard is then used consistently throughout the course of the board.

Board members use a promotion selection folder to evaluate the persons considered for promotion. The contents of the folder are determined by HQ USAF. The eligible sergeant reviews his or her selection folder and certifies as to the accuracy and completeness of the contents. A sergeant who fails to report when scheduled to review his or her selection folder is rendered ineligible for promotion consideration during that cycle unless a valid reason, such as illness or TDY, exists.

Board members assess each sergeant's folder by applying the whole-person concept. They consider such factors as manner of duty performance, experience, supervisory and leadership ability, seniority, education, and professional development. Each member makes an independent and impartial assessment of every selection in the AFSC he or she is scoring.

As indicated by table 1-1, you will be eligible for promotion to chief master sergeant after the minimum time in grade as specified.

Exercises (007):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

_____ 1. AFSC 92499 may be awarded to a master sergeant as soon as he or she has passed the USAFSE.

_____ 2. Selection to senior master sergeant and chief master sergeant is made by the same WAPS scoring system as for technical sergeant and master sergeant.

_____ 3. Promotions to senior master sergeant and chief master sergeant are based on the whole-person concept.

_____ 4. A trial run of the promotion panel is conducted to familiarize members with the evaluation process.

_____ 5. Education, professional development, and leadership ability are some of the factors considered in applying the "whole person" concept.

008. Cite requirements for promotion of feeder AFSCs for the CEM 92400 and duties of the Medical Laboratory Managers.

CEM 92400, Medical Laboratory Manager. The AFSC of Medical Laboratory Manager is awarded to all chief master sergeants and chief master sergeant selectees who have the feeder AFSC 92499, Medical Laboratory Superintendent, and AFSC 92590, Cytotechnology Superintendent, as indicated in figure 1-1. Chief master sergeants identified by the Chief Enlisted Managers (CEM) 92400, through extensive experience and training, have demonstrated managerial ability to plan, direct, coordinate, implement, and control a wide range of work activity. As a chief enlisted manager in the medical laboratory, the chief master sergeants will be subject to working in a variety of jobs and functional areas where their general managerial and supervisory abilities can be most effectively used and challenged. Some managerial duties and responsibilities of the chief are:

a. Manages and directs activities of personnel resources; interprets and enforces policy and applicable directives.

b. Establishes control procedures to meet work goals and standards.

c. Recommends or initiates actions to improve efficiency of functional operation.

d. Plans and programs work commitments and schedules.

e. Develops plans regarding procurement and maintenance of facilities, supplies, and equipment.

Chief master sergeants will be assigned a primary and control AFSC as CEM 92400. However, if the position is authorized, his or her duty AFSC will be CEM 92400.

Exercises (008):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

_____ 1. The AFSC for the Medical Laboratory Manager CEM 92400 may be awarded to senior master sergeants with extensive training and experience.

TABLE 1-2
WAPS SCORING SYSTEM

POINT FACTORS	MAXIMUM POINTS	HOW COMPUTED
Specialty Knowledge Test (SKT)	100	Use actual percent score obtained.
Promotion Fitness Examination (PFE)	100	Actual percent score obtained.
Time in Service (TIS)[1]	40	Years of active service times 2. Six months or less, one point. More than six months, two points.
Time in Grade (TIG)[2]	60	One-half point per month. Drop 15 days or less. Count more than 15 days as a month.
Decorations[3] (Credit given regardless of service in which medal was awarded.)	25	Add point value of each decoration you have been awarded. (See footnote 3.)
Airman Performance Reports (APRs)	135	APRs have scores of 0 to 9. Add all APR scores of last five years, not to exceed 10 APRs. Divide the sum by number of APRs used. This is the APR mean. Multiply by 15. This is your APR score.
	Total 460	

[1] Time in service is credited through the last month and year of the promotion cycle for which airman is being considered. [2] Time in grade is credited through first day of the last month and year of the promotion cycle for which airman is being considered. [3] Decorations scored as follows: Medal of Honor, 15; Air Force Cross, Distinguished Service Cross, 11 each; Distinguished Service Medal, Silver Star, 9 each; Legion of Merit, Distinguished Flying Cross, 7 each; Airman's Medal, Soldier's Medal, Bronze Star, and Meritorious Service Medal, 5 each; Air Medal and Commendation Medal, 3 each; Purple Heart, 1.

_____2. Both the Medical Laboratory and histopathology superintendents provide the feeder AFSCs for the Medical Laboratory Manager, CEM 92400.

_____3. One of the responsiblities and duties of the Clinical Laboratory Manager is to plan and program work commitments and schedules.

009. Name advantages and significant features of the Weighted Airman Promotion System.

Weighted Airman Promotion System. One of the main interests of all NCOs/supervisors is the subject of promotions. How can you be promoted and succeed in your career field? Let's discuss briefly the Weighted Airman Promotion System (WAPS). As you should recall, promotions to staff sergeant, technical sergeant, and master sergeant are made under WAPS. Under this system, personnel data elements are collected, validated, processed, and converted to weighted factor scores for promotion selection purposes. Table 1-2 shows the scoring system used under WAPS.

Advantages of WAPS. The WAPS is an outgrowth of several years of intensive work and study to devise an equitable promotion system that provides uniform Air Force-wide application of selection criteria. The system insures that each Air Force member receives fair and equal consideration for promotion. The WAPS also presents two other important advantages. One is what personnel people call visibility. A major irritant of past promotion systems has been the everpresent, unanswered question: "Why didn't I get promoted?" Under the WAPS, the individuals know why they are not selected, how far they missed selection, and the specific areas in which they need to improve. Another advantage is that the WAPS allows greater objectivity. Since a specified number of points are allowed in specified areas, little is left to human interpretation or subjective judgment.

WAPS test requirements. An airman eligible for promotion must take a Promotion Fitness Examination (PFE) and a Specialty Knowledge Test (SKT). The SKT for medical laboratory personnel is written by a panel of subject matter specialists who are NCOs and possess extensive experience in the field and are from various bases throughout the Air Force. Both the PFE and SKT are written to the grade level in which the airman is considered for promotion.

Selection procedures. Airmen are aligned in promotion priority order by grade, AFSC, and total weighted factor score. Those with the highest scores in each AFSC are selected to fulfill the forecasted vacancies. A list of promotion selectees is published alphabetically with priority sequence numbers established by seniority (time in grade).

Exercises (009):

1. Name three advantages of the WAPS.

2. What maximum number of points may be obtained under the WAPS for time in service?

3. What is the maximum number of points that may be obtained under the WAPS?

4. How are the APR points computed under the WAPS?

5. How many points are awarded for the Commendation Medal under the WAPS?

6. If you receive scores of 85 on your PFE and 80 on your SKT, how many WAPS points do these scores represent?

7. What three ranks are achieved under the WAPS?

010. Identify medical laboratory duties and responsibilities with the specialist, technician, and superintendent, who is responsible for each.

Duties and Responsiblities of Medical Laboratory Personnel. The airman classification structure affords the technician an opportunity to progress in the medical laboratory career field. An increase in rank will follow with a certain increase in responsibility. A summary of your job description is found in AFR 39–1. The summaries define the basic official duties and responsibilities for personnel in this career field: Medical Laboratory Specialist, AFSC 92450; Medical Laboratory Technician, AFSC 92470; and Medical Laboratory Superintendent, AFSC 92499. You will note the job descriptions of these specialties in appendixes A, B, and C.

Medical laboratory specialist. The medical laboratory specialist accomplishes standardized quantitative and qualitative evaluation of erythrocytes, leukocytes, and thrombocytes. Performs chemical analyses and macroscopic and microscopic examinations of urine specimens. Analyzes human material or other products submitted to the laboratory using chemical or physical procedures applicable to clinical chemistry. Draws and processes blood aseptically by standardized techniques. Conducts procedures necessary to isolate and identify bacteria by gross and microscopic examination, staining, biochemical, and immunological procedures. Performs microscopic and chemical examination of spinal fluid and gastric fluids. Schedules and assigns work to subordinates according to their ability and training.

Medical laboratory technician. Now let's compare the job description with that of the technician. The medical laboratory technician performs chemical analysis of all human material on products submitted to the laboratory. Accomplishes standardized quantitative and qualitative evaluation of erythrocytes, leukocytes, and thrombocytes. Conducts procedures to isolate and identify bacteria, fungi, or viruses by gross and microscopic examination. Accomplishes all techniques required for blood transfusion. Assists in research assignments in broad field of pathology. Plans and schedules work and performs and monitors laboratory quality control procedures, taking necessary action to assure appropriate precision and accuracy.

Medical laboratory superintendent. The medical laboratory superintendent designs and develops organizational structures to show lines of authority and to place responsibilities for performance of functions. Resolves technical problems pertaining to operation of medical laboratory activities. Plans and conducts on-the-job training by means of conferences, classes, lectures, and individual instruction. Conducts periodic inspections of medical laboratory activities. Assists medical officers and allied scientists in research assignments in broad field of pathology.

Exercises (010):

1. Match the job title in column B with the appropriate duty or responsibility from AFR 39–1 (Appendixes A, B, and C) in column A. Each item may be used more than once, and more than one column B may match a single column A entry.

Column A

______ (1) Designs and develops organizational structures to show lines of authority.
______ (2) Accomplishes standardized quantitative and qualitative evaluation of erythrocytes.
______ (3) Plans and conducts on-the-job training.
______ (4) Performs and monitors laboratory quality control procedures.

Column B

a. Medical laboratory specialist.
b. Medical laboratory technician.
c. Medical laboratory superintendent.

Column A

______ (5) Conducts periodic inspections of medical laboratory activities.
______ (6) Evaluates duty performance.
______ (7) Evalutes work performed, effectiveness training.
______ (8) Accomplishes general laboratory duties.
______ (9) Assists in blood bank duties.
______ (10) Assists in identification of viruses and fungi.
______ (11) Prepares tissue for electron microscopy.
______ (12) Directs continuous organized training programs to keep personnel current on new or revised techniques, policies, and procedures.

CHAPTER 2

Laboratory Materiel and General Laboratory Safety

EVERY LABORATORY needs supplies. As the seven-level technician, you should have a thorough knowledge and understanding of the way in which laboratory supplies and equipment are obtained. You should also know how the proper stock levels are maintained in order to insure an efficiently operated and cost effective laboratory.

Likewise, maintaining good safety techniques and procedures is as important as producing good quality laboratory tests results. With this in mind, you will also review safety procedures, first aid, and accident reporting in clinical chemistry.

2-1. Medical Supply Activities

You must have a thorough knowledge of the supply system, since you will frequently sign for new equipment, turn in old equipment, and act as the responsible property officer for the equipment in your laboratory. As a laboratory supervisor, you must be familiar with:

a. What items are required, when, how often, in what quantities, and how they are ordered.

b. How to coordinate your supply problems with supply personnel in order to remain informed about overdue orders, problem areas, and policy changes.

c. How to maintain efficient records of all supply transactions.

d. How to provide an efficient system for the storage and security of supplies and equipment.

This section is designed to assist you with these procedures.

011. Differentiate among the six categories of medical materiel.

Classification of Medical Materiel. Medical materiel may be in the form of equipment or supplies. Equipment items are nonexpendable and must be authorized. Supplies are expendable and need no authorization. Materiel with which you should be familiar is classified into one of the three equipment and two supply categories described below.

Investment medical equipment. Investment medical equipment denotes those items with a unit cost of $3,000 or more and a life expectancy of 5 years or longer. Some examples are biological safety cabinet and glassware dryer.

Expense medical equipment. An item classified as expense medical equipment is one with a unit cost of at least $300, but less than $3,000, and a life expectancy of at least 5 years.

Nonmedical equipment. Nonmedical equipment denotes those items that meet the criteria established in AFM 67–1, *USAF Supply Manual*, Volume 4, Part 1. Further, equipment management codes (EMC) that are assigned nonmedical equipment in TA 001 and guidance provided in AFM 67–1, Volume 2, part 2, Chapter 22, will be used to determine which items require accountable records. Both volumes are normally maintained at Base Supply. Thus, nonmedical equipment cannot be classified on the basis of an assigned dollar value.

Expendable medical supplies. Expendable medical supplies are consumable and durable. A consumable supply item loses its identity when used, cannot be reused for the same purpose, or is not durable enough to last 1 year. Reagents, disposable needles, syringes, and adhesive tape are examples. A durable supply item maintains its identity when used, usually has a life expectancy of at least 1 year, but does not qualify as an equipment item. Instruments such as scissors and hemostats are examples of durable supplies.

Nonmedical supplies. These supplies are items that are nonmedical in nature. Some examples are pencils, ballpoint pens, paper towels, typing paper, and typewriter ribbons.

Exercises (011):

1. Match the materiel category in column B with the appropriate statement or term in column A. Each column B item may be used once or more than once. In addition, one or more column B items may match a single column A entry.

Column A	*Column B*
_____(1) A $14,000 automated sodium and potassium analyzer with life expectancy of 5 years.	a. Investment medical equipment. b. Expense medical equipment. c. Nonmedical equipment. d. Expendable medical supplies (consumable).
_____(2) A $2,500 Bright-Field microscope with a life expectancy of 5 years.	e. Expendable medical supplies (durable). f. Nonmedical supplies.
_____(3) Ten boxes of pinworm paddles valued at $385.20.	

Column A

______(4) Box of paper clips.

______(5) Cannot be classified on the basis of an assigned dollar value.

______(6) Life expectancy of at least 5 years or more.

______(7) Guidance is provided in AFM 67-1, Volume 2, Part 2, Chapter 22, to determine which of such items require accountable records.

______(8) Supply items that lose their identity when used.

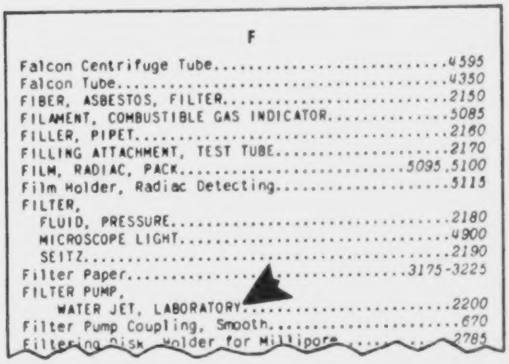
______(9) One box of 20 gauge vacutainer needles.

______(10) One box of hemostats to be used in blood bank.

______(11) Ultra-low temperature freezer, $5,995.

012. Identify supply classification, identification terms, and supply catalogs as they relate to the medical laboratory.

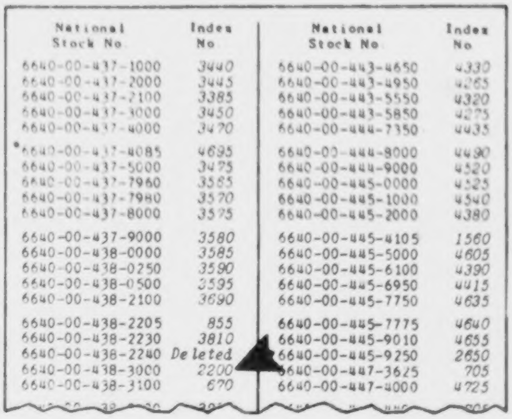
Item Classification and Identification. In the Federal supply system, a group of items is classified by the Federal Supply Classification (FSC), which is the first four digits of the stock number. Each class of items is classified by the general category name and number. Examples: 6505, Drugs, Biological, and Official Reagents; 6510, Surgical Dressing Materials; 6515, Medical—Surgical Instruments, Equipment, and Supplies; and FSC Group 66, Instruments and Laboratory Equipment.



F

Falcon Centrifuge Tube............................4595
Falcon Tube.......................................4350
FIBER, ASBESTOS, FILTER...........................2150
FILAMENT, COMBUSTIBLE GAS INDICATOR...............5085
FILLER, PIPET.....................................2160
FILLING ATTACHMENT, TEST TUBE.....................2170
FILM, RADIAC, PACK...........................5095,5100
Film Holder, Radiac Detecting.....................5115
FILTER,
  FLUID, PRESSURE.................................2180
  MICROSCOPE LIGHT................................4900
  SEITZ...2190
Filter Paper.................................3175-3225
FILTER PUMP,
   WATER JET, LABORATORY..........................2200
Filter Pump Coupling, Smooth.......................670
Filtering Disk, Holder for Millipore..............2785

Figure 2–1. Sample NSN Alphabetical Index.



National Stock No	Index No	National Stock No	Index No
6640-00-437-1000	3440	6640-00-443-4650	4330
6640-00-437-2000	3445	6640-00-443-4950	4265
6640-00-437-2100	3385	6640-00-443-5550	4320
6640-00-437-3000	3450	6640-00-443-5850	4275
6640-00-437-4000	3470	6640-00-444-7350	4435
•6640-00-437-4085	4695	6640-00-444-8000	4490
6640-00-437-5000	3475	6640-00-444-9000	4520
6640-00-437-7960	3565	6640-00-445-0000	4525
6640-00-437-7980	3570	6640-00-445-1000	4540
6640-00-437-8000	3575	6640-00-445-2000	4380
6640-00-437-9000	3580	6640-00-445-4105	1560
6640-00-438-0000	3585	6640-00-445-5000	4605
6640-00-438-0250	3590	6640-00-445-6100	4390
6640-00-438-0500	3595	6640-00-445-6950	4415
6640-00-438-2100	3690	6640-00-445-7750	4635
6640-00-438-2205	855	6640-00-445-7775	4640
6640-00-438-2230	3810	6640-00-445-9010	4655
6640-00-438-2240	Deleted	6640-00-445-9250	2650
6640-00-438-3000	2200	6640-00-447-3625	705
6640-00-438-3100	670	6640-00-447-4000	4725

Figure 2–2. Sample National Stock Number Index.

Identification Lists (ILs). Identification Lists are published or grouped according to the Federal Supply Classification (FSC) number; for example, Identification List, FSC Group 66, Publication Number C–6600–IL, Instrument and Laboratory Equipment. These identification indices identify items by name, picture, and an accompanying description along with the national stock number. Each identification list also contains an alphabetical index and a stock number index, which may be used for cross-referencing items. This publication provides the data described below.

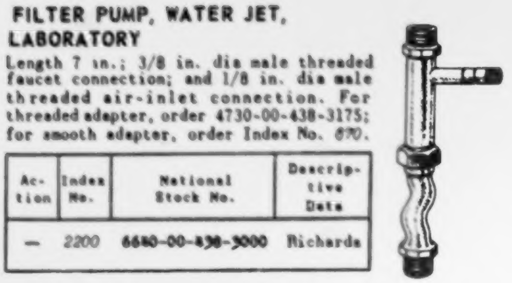
Alphabetical index. This is a list (fig. 2-1) in alphabetical sequence of item names, synonyms, colloquials, common names, and trademark names referenced to index numbers to assist in locating an item within the subsection. Item names appear in uppercase letters; other names appear in uppercase and lowercase letters. The alphabetical index in FSC Class 6505 contains only approved item names referenced to their applicable index numbers. Colloquials, synonyms, and trademark names are referenced to their appropriate approved item names in the "Glossary of Colloquial Names and Therapeutic Index, C–6505–GL."

National stock number index. This is a list of national stock numbers arranged in numerical sequence referenced to applicable index numbers to assist in locating an item within the subsection (figs. 2-2 and 2-3). Note that the arrow in figure 2-2 points to the index number of the same item in figure 2-3. Items that have been deleted since the dates of the superseded publication are marked "Deleted" in the Index No. column.

Identification data. The following data are arranged alphabetically by the item name under which the item is identified in the Federal cataloging system (fig. 2-3). Each item or a representative item is illustrated when such presentation is necessary for identification.

a. Action code—denotes an addition, deletion, or revision of published data, as follows:

(1) N (New)—indicates an item not previously included in the basic publication, change bulletin, or change notice,



FILTER PUMP, WATER JET, LABORATORY

Length 7 in.; 3/8 in. dia male threaded faucet connection; and 1/8 in. dia male threaded air-inlet connection. For threaded adapter, order 4730-00-438-3175; for smooth adapter, order Index No. 870.

Action	Index No.	National Stock No.	Descriptive Data
—	2200	6640-00-438-3000	Richards

Figure 2-3. Sample identification list entry.

or a reinstatement of an item previously deleted from a basic publication, change bulletin, or change notice.

(2) C (Change)—indicates a change in data since the previous publication.

(3) D (Deletion)—indicates that an item has been deleted.

b. Index numbers—items are presented in alphabetical order; index numbers are assigned in ascending sequence within each pamphlet. The index numbers are used solely as a locator device and are NOT to be used in lieu of the national stock number nor to requisition materiel. Upon revision of a catalog subsection, the index numbers are revised to conform with the sequence of items.

c. National stock number—the 11-digit Federal stock number has been changed to the 13-digit national stock number in medical catalogs. Two digits have been added to denote the NATO code number. The first four digits comprise the Federal Supply Classification (FSC), and the last nine digits comprise the national item identification number (NIIN), which uniquely identifies the item (fig. 2-4). The 13-digit NSN is used to order items. An item is identified by both name and number.

d. Descriptive data—important distinguishing characteristics are stated in the Descriptive Data column.

e. Description—appears below the item name and above the box in which the Index No., National Stock Number, and Descriptive Data are arranged; or may be arranged in an additional column to the right of the Descriptive Data column. Operational information may appear as a footnote below the boxed data.

f. Information regarding special storage and handling of an item appears under "Notes." The symbols used are shown in table 2-1.

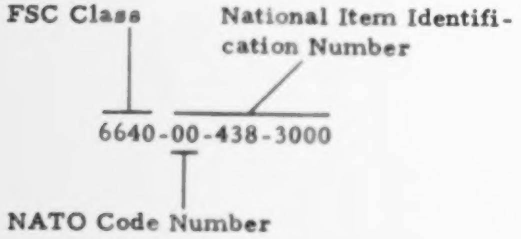


Figure 2-4. The national stock number (NSN).

TABLE 2-1

NATIONAL SUPPLY SYSTEM NOTE

Symbol	*Explanation*
D	Subject to deterioration in a period of 36 months or less.
F	Subject to damage by freezing.
G	Requires refrigeration between 2°C and 8°C (35°F to 46°F).
I	Flammable or oxidizing.
K	An item which is a drug, or other substance determined by the Director, Bureau of Narcotics and Dangerous Drugs, Department of Justice, to be designated Schedule Symbol III, IV, or V, as defined in the Controlled Substances Act, effective 1 May 1971, and other items requiring security storage.
P	Item with potency period or expiration date.
R	Alcohol, alcoholic beverages, precious metals, or a drug or other substance determined by the Director, Bureau of Narcotics and Dangerous Drugs, Department of Justice, to be designated Schedule Symbol II, as defined in the Controlled Substances Act, effective 1 May 1971, and other items requiring vault storage.
W	Must be frozen for preservation.

Management list (ML). The management list index (fig. 2-5) lists supply management data by national stock number. This supply management data includes:

a. An action code, identifying an action not previously published. These codes are shown in table 2-2.

b. An acquisition advice code, indicating how an item will be acquired in the Defense Medical Supply System. Table 2-3 shows the acquisition advice codes, together with their meanings. In addition to acquisition advice codes and stock number, figure 2-5 shows:

c. Price.

d. Unit of issue—for example, pair, each, roll, set.

e. Packaging and packing information, including (1) intermediate package quantity and (2) exterior container quantity.

f. Weight and cubage.

When your requirements approximate the intermediate package or exterior container quantities, requisition these quantities, since this eliminates the need to repackage for shipping.

Action	Acquisition Advice Code	National Stock No.	Price	Unit of Issue	Packaging & Packing		Weight and Cubage			
					Intermediate Package Qty	Exterior Container Qty	Unit of Issue Weight	Exterior Container Weight	Unit of Issue Cube	Exterior Container Cube
	D	6640-00-434-5000	5.42	EA	1	24	2.3585	57	.0925	2.220
	D	6640-00-435-0379	17.50	PG	1	5	5.6000	26	.0752	.376
	D	6640-00-435-5200	45.40	SE	1	12	7.3333	88	.2667	3.200
	D	6640-00-435-5400	13.70	SE	12	216	.3261	70	.0196	4.234
	V	6640-00-435-5645	42.60	EA	1	4	14.0000	56	1.0000	4.000
	D	6640-00-438-3000	5.24	EA	1	48	1.5000	72	.0458	2.200
	D	6640-00-438-3100	1.20	EA	12	288	.2090	60	.0022	.634
	V	6640-00-438-5000	.39	EA	12	288	.6528	188	.0400	11.500
	D	6640-00-438-5950	1.30	PG	12	48	1.3542	65	.0333	1.600
C	D	6640-00-438-9600	139.00*	EA		1	52.0000	52	3.0000	3.000

Figure 2–5. Sample management data list entries.

Federal supply catalogs. The identification list and the management list are published separately, but used together they may be considered as a Federal supply catalog. For the medical laboratory, the three most commonly used Federal supply catalogs are: C–6505/6508–IL, Drugs, Biologicals, and Official Reagents; C6600–IL, Instruments and Laboratory Equipment; and C–6700/9500–IL (FSC Class 6810 CHEMICALS).

Another Federal supply catalog frequently used is the General Services Administration (GSA) catalog. The GSA catalog contains information on many office and expendable supplies used throughout the Federal Government. These three catalogs may be researched for specific items of equipment or supplies required in the medical laboratory.

TABLE 2-2
NSN ACTION CODES

Code	Action
C	Indicates a change in data other than the National Stock Number and is identified by an asterisk to the right of the data changed.
D	Indicates a National Stock Number has been deleted and not replaced.
N	Indicates a National Stock Number not previously included in the basic publication or change bulletin, or reinstatement of a National Stock Number.
R	Indicates that a National Stock Number has been deleted and replaced, or changed.

Civilian supply catalogs. When you are unable to find a specific item in one of the Federal supply catalogs, it may then be necessary to use a civilian catalog. You should not procure items from civilian catalogs if a satisfactory item is available through the Federal Supply system. This is because items ordered through a civilian catalog are more expensive than they would be if they could be ordered via the Federal Supply System. You will find out later in this volume that the same basic procedures apply for ordering through a civilian catalog as for the Federal supply catalogs.

Exercises (012):

1. Match each supply term in column B with the appropriate description in column A.

Column A

_____(1) FSC for Medical—Surgical Instruments, Equipment, and Supplies.
_____(2) FSC for Drugs, Biologicals, and Official Reagents.
_____(3) FSC for Instruments and Laboratory Equipment.
_____(4) FSC for Surgical Dressing Materials.
_____(5) Identify items by name, picture, and description.
_____(6) Lists item names, synonyms, colloquial names, common names, and trademark names in alphabetical sequence.

Column B

a. Action code.
b. Identification lists.
c. FSC Group 6505.
d. FSC Group 66.
e. FSC Group 6510.
f. FSC Group 6515.
g. Alphabetical index.
h. National stock number.
i. Index numbers.
j. Information appearing under "Notes."
k. Management list (ML).
l. Civilian supply catalogs.
m. Federal supply catalog.
n. GSA catalog.

Column A

______(7) Numerical list of national stock numbers.
______(8) Denotes an addition, deletion, or revision of published data.
______(9) Information regarding special storage and handling of an item.
______(10) Lists supply management data by national stock number.
______(11) Identification list and management list used together.
______(12) Contains information on office and expendable supplies.
______(13) Used when an item does not appear in the Federal supply catalogs.
______(14) Items are listed in alphabetical order; index numbers are assigned in ascending sequence; not to be used in place of NSN to requisition materiel.

013. Cite procedures for making supply transactions for request, turn-in, and maintenance of records pertaining to medical supply items.

Ordering Supplies. We will now examine the two methods of ordering supplies. Keep in mind that regardless of the method used, if you order an item, it must appear on the issue list that you receive when the supplies are delivered. This is especially important in case an item is "back ordered." The back-ordered item must appear on the issue list, or you will never receive the item from Supply when they receive it from the depot. Report the omission to Medical Supply.

Telephone orders. This is the preferred method of requesting supplies because of its speed and simplicity. The medical supply account provides you with a shopping guide. This is a computer-produced listing, which shows all the expendable items that you order on a recurring basis. The items are listed in stock number sequence, with each item having a line number. The unit of issue, item nomenclature, and unit price are also shown for each item. The medical supply account maintains a shopping guide card for each item listed on your shopping guide.

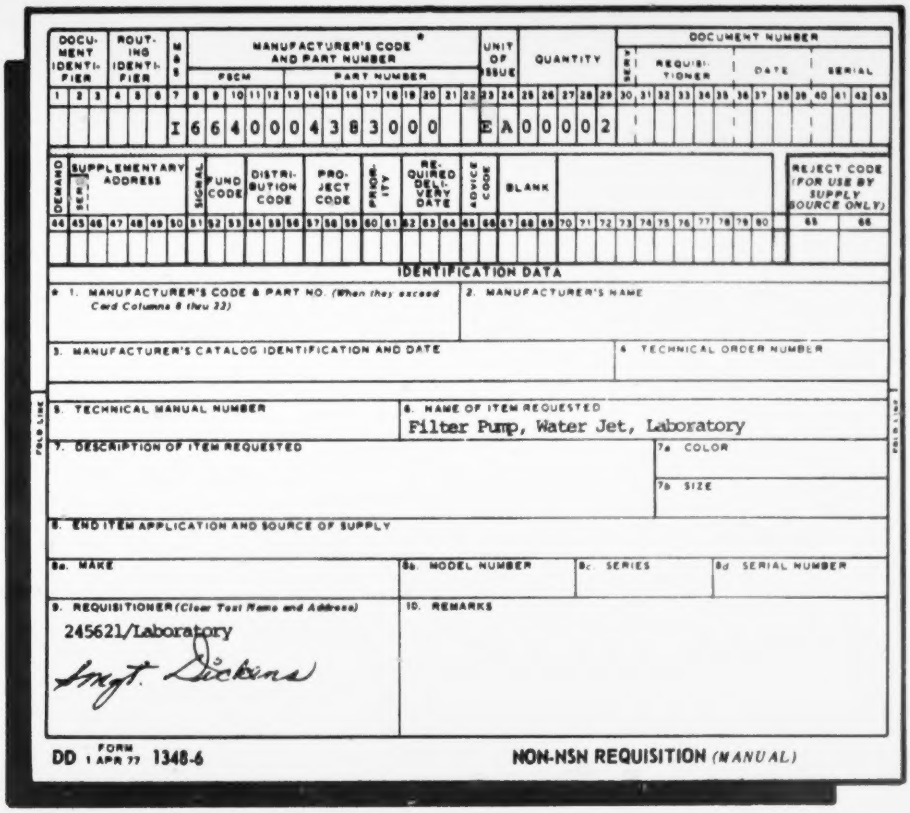
When it's time for you to order, call Medical Supply, and using your shopping guide, place your order by line number. For example, "Line 4, six bottles; line 6, two boxes; line 9, one can." As you order, the medical supply clerk pulls the appropriate card from the file and annotates the quantity requested on the card. You can see why this is the preferred method: it saves time at both ends of the supply line.

TABLE 2-3
NSN ACQUISITION ADVICE CODES

Code	*Acquisition Advice*
A	Service regulated item. The use and storage of this item requires release authority from the appropriate medical service. Requisitions will be submitted in accordance with the military services' requisitioning procedures.
D	Item centrally managed, stocked and issued under control of one Inventory Control Point (ICP) for all customers.
H	Item centrally procured but not stocked.Issue is by direct shipment from the vendor to the user at the order of the ICP. (Except where a military service directs otherwise, MILSTRIP requisitions will be submitted directly to the Directorate of Medical Materiel, Defense Personnel Support Center, 2800 South 20th Street, Philadelphia PA 19101. Requisitions should provide for sufficient procurement lead time [at least 90 days after receipt of requisitions at DPSC].)
L	Item authorized for local purchase, as a normal means of support, at base, post, camp, or station level. Item not stocked in domestic depot system. Appeal to ICP, or to prescribed purchasing activity, when unable or not permitted to procure locally.
V	Item in stock; but future procurement is not authorized. Requisitions may continue to be submitted until stocks are exhausted.
W	Restricted requisitioning. Stock number has been assigned for use in bid invitations. Requisitions will not be submitted to DPSC until otherwise advised by the military services.
X	Semiactive item; item not authorized for stockage at wholesale level.

DD Form 1348–6, Non-NSN Requisition (Manual). When you require an expendable item that is not listed on your shopping guide, you must submit your order on DD Form 1348–6 (fig. 2-6). If you wish to have the item added to your shopping guide, write in boldface letters on the face of the DD Form 1348–6: "ADD TO SHOPPING GUIDE."

Standard item less than $300. A standard item is one that is listed in Federal supply catalogs. Normally, no justification is required, and the items are ordered on DD



DOCUMENT IDENTIFIER	ROUTING IDENTIFIER	M & S	MANUFACTURER'S CODE AND PART NUMBER * — FSCM / PART NUMBER	UNIT OF ISSUE	QUANTITY	DOCUMENT NUMBER — SERV / REQUISITIONER / DATE / SERIAL
1 2 3	4 5 6	7	8 9 10 11 12 13 14 15 16 17 18 19 20 21 22	23 24	25 26 27 28 29	30 31 32 33 34 35 36 37 38 39 40 41 42 43
		I	6640004383000	EA	00002	

DEMAND	SUPPLEMENTARY ADDRESS (SERV)	SIGNAL	FUND CODE	DISTRIBUTION CODE	PROJECT CODE	PRIORITY	REQUIRED DELIVERY DATE	ADVICE CODE	BLANK		REJECT CODE (FOR USE BY SUPPLY SOURCE ONLY)
44	45 46 47 48 49 50	51	52 53	54 55 56	57 58 59	60 61	62 63 64	65 66	67 68 69	70 71 72 73 74 75 76 77 78 79 80	65 66

IDENTIFICATION DATA

* 1. MANUFACTURER'S CODE & PART NO. *(When they exceed Card Columns 8 thru 22)*

2. MANUFACTURER'S NAME

3. MANUFACTURER'S CATALOG IDENTIFICATION AND DATE

4. TECHNICAL ORDER NUMBER

FOLD LINE

5. TECHNICAL MANUAL NUMBER

6. NAME OF ITEM REQUESTED: Filter Pump, Water Jet, Laboratory

7. DESCRIPTION OF ITEM REQUESTED

7a COLOR

7b SIZE

8. END ITEM APPLICATION AND SOURCE OF SUPPLY

8a. MAKE

8b. MODEL NUMBER

8c. SERIES

8d SERIAL NUMBER

9. REQUISITIONER *(Clear Text Name and Address)*: 245621/Laboratory
Sngt. Dickens

10. REMARKS

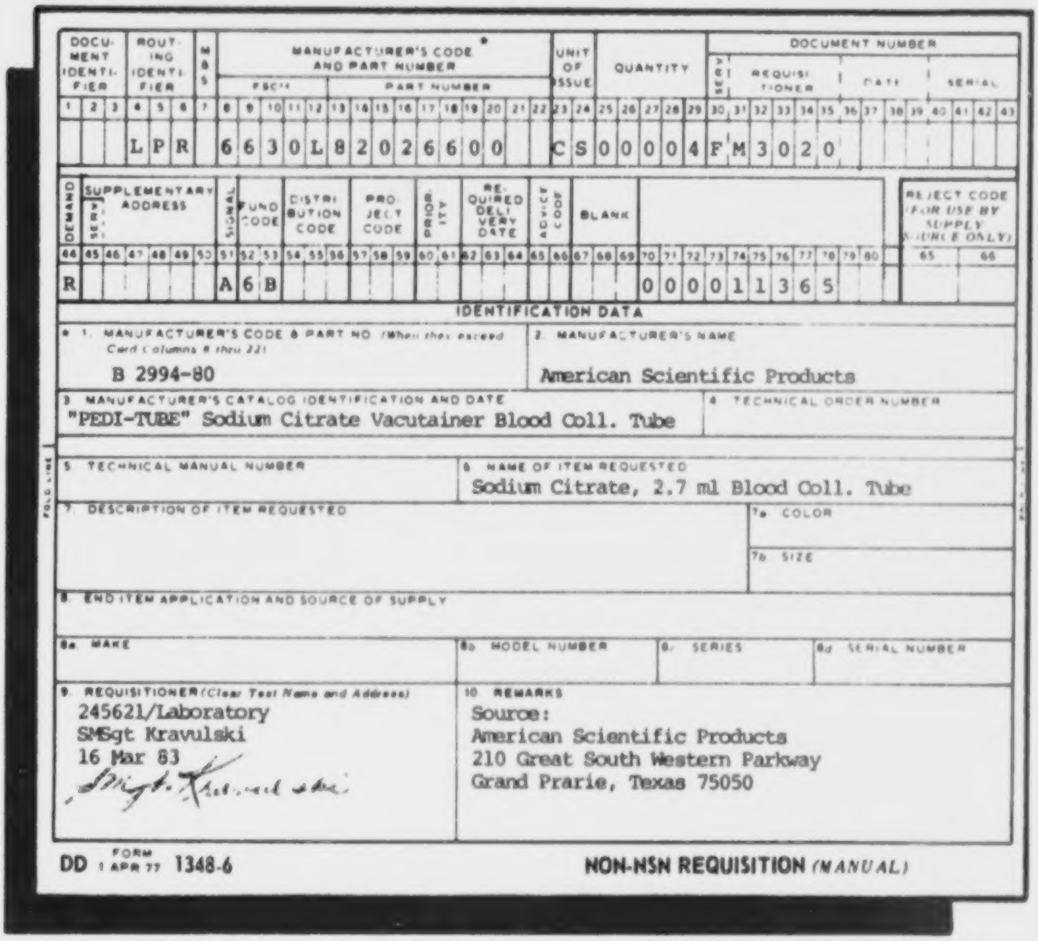
DD FORM 1 APR 77 1348-6

NON-NSN REQUISITION *(MANUAL)*

The following basic information should be completed by the Laboratory Technican when preparing DD Form 1348-6, for standard item requisitions:

Columns	
4-6	Indicate "LPR" if local purchase, if not, leave blank.
7	"I" for issue and "T" for turn-in.
8-22	Stock number asigned to the item.
23-24	Unit of issue.
25-29	Quantity.
30-35	Leave blank.
36-39	Leave blank.
40-43	Leave blank.
44-69	Leave blank.
Block	
6	Nomenclature.
7	a. Using activity or account code.
	b. Signature of individual receiving materiel if required.

Figure 2-6. Sample of DD Form 1348-6.



DOCUMENT IDENTIFIER	ROUTING IDENTIFIER	M&S	MANUFACTURER'S CODE AND PART NUMBER * — FSCM	PART NUMBER	UNIT OF ISSUE	QUANTITY	DOCUMENT NUMBER — SERV / REQUISITIONER	DATE	SERIAL
1 2 3	4 5 6	7	8 9 10 11 12	13 14 15 16 17 18 19 20 21 22	23 24	25 26 27 28 29	30 31 32 33 34 35	36 37 38 39	40 41 42 43
	LPR		6630L	82026600	CS	00004	FM3020		

DEMAND	SUPPLEMENTARY ADDRESS	SIGNAL	FUND CODE	DISTRIBUTION CODE	PROJECT CODE	PRIORITY	REQUIRED DELIVERY DATE	ADVICE CODE	BLANK		REJECT CODE (FOR USE BY SUPPLY SOURCE ONLY)
44	45 46 47 48 49 50	51	52 53	54 55 56	57 58 59	60 61	62 63 64	65 66	67 68 69	70 71 72 73 74 75 76 77 78 79 80	65 66
R		A	6B							000011365	

IDENTIFICATION DATA

* 1. MANUFACTURER'S CODE & PART NO (When they exceed Card Columns 8 thru 22)
B 2994-80

2. MANUFACTURER'S NAME
American Scientific Products

3. MANUFACTURER'S CATALOG IDENTIFICATION AND DATE
"PEDI-TUBE" Sodium Citrate Vacutainer Blood Coll. Tube

4. TECHNICAL ORDER NUMBER

FOLD LINE

5. TECHNICAL MANUAL NUMBER

6. NAME OF ITEM REQUESTED
Sodium Citrate, 2.7 ml Blood Coll. Tube

7. DESCRIPTION OF ITEM REQUESTED

7a. COLOR

7b. SIZE

8. END ITEM APPLICATION AND SOURCE OF SUPPLY

8a. MAKE

8b. MODEL NUMBER

8c. SERIES

8d. SERIAL NUMBER

9. REQUISITIONER (Clear Text Name and Address)
245621/Laboratory
SMSgt Kravulski
16 Mar 83

10. REMARKS
Source:
American Scientific Products
210 Great South Western Parkway
Grand Prarie, Texas 75050

DD FORM 1 APR 77 1348-6

NON-NSN REQUISITION *(MANUAL)*

Figure 2-7A. Sample of DD Form 1348-6, Non-NSN Requisition (Manual).

Form 1348-6. Because of recent changes, the DD Form 1348-6 may be used for both standard and nonstandard items less than $300.

Standard items over $300. A justification is always required for items over $300; these items are ordered on AF Form 601, Equipment Action Request. Instructions for completing may be obtained from the medical materiel department.

Nonstandard items less than $300. Nonstandard items are those NOT found in Federal supply catalogs, and must be locally purchased. Depending upon local policy, a justification for such items may or may not be required. In either case, though, nonstandard items less than $300 are ordered on DD Form 1348-6, Non-NSN Requisition (Manual). This will frequently include nonstocklisted replacement parts for stocklisted equipment.

Nonstandard items over $300 and nonstandard replacement parts for nonstandard equipment. These items must be ordered on an AF Form 601, Equipment Action Request. You may also have to submit a DD Form 1348-6 when required at some facilities. A sample copy of this form and instructions for preparing it for medical items are shown in figures 2-7A and figure 2-7B.

Turn-in procedures. When supplies become excess to the needs of a using activity or become unserviceable, as the property custodian you may turn in the surplus to the Medical Equipment Management Office (MEMO). Certain items may be transferred to another activity that may need them. If a replacement item is required for an unserviceable one, a requisition (AF Form 601) should be submitted for the new item.

			Identification Data Section:		
Card Columns	Title	Entry	Item	Title	Entry
1 - 3	Document Identifier.	"AOE" for CONUS; "A05" for O-sea.	1	Manufacturer's code and part No.	Enter if applicable.
4 - 6	Routing Identifier.	Enter "LFR".	2	Manufacturer's name.	Manufacturer's name and address. If more than one, enter in remarks section. Enter "OR EQUAL" if additional manufacturers not given. If item is acceptable from only one manufacturer, attach a sole source justification.
7		Enter "I" for issue.			
8 - 22	Manufacturer's code and part No.	Enter LSN if known; otherwise leave blank.			
23 - 24	Unit of Issue.	Self-explanatory.			
25 - 29	Quantity.	Self-explanatory; use zeros to fill up spaces.	3	Manufacturer's catalog ID and date.	Enter catalog number and date if known.
30 - 35	Requisitioner.	Enter FM code.	4	Technical Order No.	If applicable.
36 - 39	Document date.	Leave blank.	5	Technical Manual No.	Leave blank.
40 - 43	Document serial No.	Leave blank.	6	Name of item.	Self-explanatory.
44	Demand code.	Enter "R" for recurring, or "N" for nonrecurring.	7	Description of item.	Be complete.
45 - 50	Supply address.	Leave blank.	8	End item application and source of supply.	Include the NSN of the end item if you are ordering spare parts; also include manufacturer, make, model, etc.
51	Signal code.	Enter "A".			
52 - 53	Fund code.	Enter "6B".			
			9	Requisitioner.	Enter using activity account code, initiator's name, date material is required, and signature of individual receiving materiel.
60 - 61	Priority designator.	Leave blank.			
71 - 78	Estimated unit price.	Enter estimated price of item to nearest cent. Do not use total cost of item (exclude shipping, etc.)	10	Remarks section.	Source and other data, if needed.
79 - 80	Unit price.	Leave blank.			

Figure 2-7B. Sample of DD Form 1348-6, instructions.

Equipment is nonexpendable and, like supplies, is either medical or nonmedical, new or replacement. Replacement of equipment depends upon present condition, life expectancy, maintenance costs, and other priorities.

When submitting AF Form 601 with the equipment that is deemed unserviceable, you should mention the condition code as determined by the medical equipment repair technician and the life expectancy of the equipment. Examples of source codes are found in figures 2-8 and 2-9. Condition code information is obtained from AFM 67-1, Volume 5, Chapter 19, Atch 3, USAF Supply Manual, and the life expectancy information is obtained from Atch B-4 (Part 1) of the same Air Force manual. The property custodian will receive a custody receipt locator list and a custodial action list at specified times. Custody receipt locator list is received at least annually. It is used for conducting the MEMO inventory of all equipment items assigned to the property custodian: It has a threefold use: (1) annual inventory, (2) when changing property custodians, and (3) when there have been numerous transactions processed against the account.

The Custodial Actions List reflects any changes to the equipment records of the property custodian. Essentially, the Custodial Actions List is useful as an update to the Custody Receipt Locator List.

The Using Activity Back Order Report reflects the current status of equipment due-ins/due-outs for both supplies and equipment.

Code			Brief Definition	Expanded Definition
MER	GSA	AFMMFO		
A	N-1	H	New--Excellent	New or unused property in excellent condition. Ready for use and identical or interchangeable with new items delivered by a manufacturer or normal source of supply.
BF	N-2	I	New--Good	New or unused property in good condition. Does not quite qualify for condition code "N-1" (because slightly shopworn, soiled, or similar), but condition does not impair utility.
CL	N-3	J	New--Fair	New or unused property in fair condition. Soiled, shopworn, rusted, deteriorated, or damaged to the extent that utility is slightly impaired.
MR	N-4	*	New--Poor	New or unused property so badly broken, soiled, rusted, mildewed, deteriorated, damaged, or broken that its condition is poor, and its utility seriously impaired.
S	E-1	K	Used--Reconditioned Excellent	Used property but repaired or renovated and in excellent condition.
S	E-2	L	Used--Reconditioned Good	Used property which has been repaired or renovated and, while still in good usable condition, has become worn from further use and cannot qualify for excellent condition.
S	E-3	M	Used--Reconditioned Fair	Used property which has been repaired or renovated but has deteriorated since reconditioning and is only in fair condition. Further repairs or renovation required or expected to be needed in near future.
S	E-4	*	Used--Reconditioned Poor	Used property which has been repaired or renovated and is in poor condition from serious deterioration; that is, from major wear-and-tear, corrosion, exposure to weather, or mildew.
A	O-1	N	Used--Usable without repairs--Excellent	Property which has been slightly or moderately used, no repairs required, and still in excellent condition.
BF	O-2	O	Used--Usable without repairs--Good	Used property, more worn than condition code "O-1," but still in good condition with considerable use left before any important repairs would be required.
CL	O-3	P	Used--Usable without repairs--Fair	Used property which is still in fair condition and usable without repairs; however, somewhat deteriorated, with some parts (or portion) worn and should be replaced.
MR	O-4	*	Used--Usable without repairs--Poor	Used property which is still usable without repairs but in poor condition and undependable or uneconomical in use. Parts badly worn and deteriorated.
Y	R-1	Q	Used--Repairs required--Excellent	Used property, still in excellent condition, but minor repairs required (estimated repairs would cost not more than 10 percent of the standard price).
Y	R-2	R	Used--Repairs required--Good	Used property, in good condition but considerable repairs required. Estimated cost of repairs would be from 11 percent to 25 percent of the standard price.

Figure 2-6. Sample of condition codes.

Item	Life Expectancy (In Years)
Federal Supply Group 66	
Analyzing kit, meat fat content	6
Balances:	
Analytical	12
Prescription	9
Torsion	10
Blood gas apparatus	5
Blood testing kit, carbon monoxide	5
Case, microscopy supply set	9
Centrifuges	12
Comparators, color	6
Counter, blood cells	9
Counting apparatus, bacterial colony	9
Crucibles, ignition	8
Demineralizers	7
Detectors, hydrogen sulfide	6
Distilling apparatus	9
Ice making machines, laboratory	9
Incubators, bacteriological	9
Indicators:	
Carbon monoxide	8
Combustible gas	8
Lights, microscope	9
Meter, titration	7
Meters:	
Air velocity	9
Hydrogen ion test	10
Microscopes	11
Microscopy supply set	10
Microtomes	9
Mixing chamber, electric food blender	10
Ovens, laboratory	10
Photometers, flame	10
Pipetting machines	6
Refractometer, hand	10
Scale, dial indicating	12
Shaking machines, laboratory	5
Spectrophotometers	10
Stirrer-hot plates, magnetic	9
Test sets, water turbidity and color	7
Tissue processors	12
Titrator	9
Washer, pipet	8
Washing machine, glassware	8
Water baths, electric, serological	9
Water testing kits, bacteriological	7

Figure 2–9. Sample of equipment life expectancy.

Exercises (013):

Indicate whether each of the given statements is true (T) or false (F) and correct those that are false.

_____ 1. All supplies delivered to you do not have to appear on your issue list.

_____ 2. Ordering by telephone is considered the preferred method of requesting supplies because of speed and simplicity.

_____ 3. When you require an expendable item not listed on your shopping guide, you must submit your request on DD Form 1348–6.

_____ 4. Justification is required for all items less than $300 ordered on DD Form 1348–6.

_____ 5. Justification is required for all standard items over $300 on AF Form 601, Equipment Action Request.

_____ 6. Some nonstandard items are listed in Federal supply catalogs.

_____ 7. Nonstandard replacement parts over $300 for nonstandard equipment must be ordered on AF Form 1348–6.

_____ 8. When supplies become excess or unserviceable, they should be turned in to the medical equipment repair office.

_____ 9. Replacement of equipment depends upon present condition, life expectancy, maintenance costs, and other priorities.

_____ 10. When submitting unserviceable equipment on the AF Form 601, it is only necessary to mention the life expectancy.

_____ 11. Condition code information is obtained from AFM 67–1, *USAF Supply Manual* Volume 5.

_____ 12. The Custody Receipt Locator List is used for conducting the MEMO inventory of all equipment items assigned to the property custodian.

_____ 13. The Using Activity Back Order Report reflects the update of the Custody Receipt Locator List.

_____ 14. Life expectancy information is also found in AFM 67–1, *USAF Supply Manual*, Attachment B–4, Part 1.

014. Cite two reasons for using stock levels, and state how stock levels are established and maintained.

Stock Levels. Supplies are usually ordered every week or as directed by local supply policies. Let us assume that you are the supply person in your section and that you must establish levels on some new supply items. Since you usually order every week, you must check the consumption rate of these items between ordering periods. After the amount used between ordering periods is determined, you can establish a level. You are authorized a maximum 2-week level, based on normal usage for recurring-demand, expendable supplies. This level helps to insure that the items are on hand when they are needed. Another reason for establishing levels is to prevent an accumulation of excess supplies, which take up storage space, require more handling, and tie up money unnecessarily.

Remember that the level that Medical Supply maintains for an item is directly related to your consumption rate. For the system to work, your consumption rate should be consistent with your ordering rate. You order from Medical Supply; Medical Supply issues the item to you and, in turn, orders replacement materiel. This should help you understand why it is so important for you, the user, to establish realistic levels. For example, suppose that you use four boxes of vacutainer needles each week. You would then establish a 2-week level of eight boxes. Using your weekly consumption as a guide, the medical supply computer establishes a medical supply level for the item. This level is automatically reviewed and adjusted by the computer each month. Suppose that, because you have "stockpiled" vacutainer needles, you do not order the item for 3 months. If you haven't ordered the item for 3 months, you will probably ask for a large order when you do submit the request. The computer may assume that Medical Supply must replace all those boxes of vacutainer needles you have just ordered and generate a requisition to the supply depot to do just that. Now you have a stockpile of vacutainer needles in your department, and Medical Supply also has a stockpile of the vacutainer needles sitting in the warehouse.

The computer may also react differently if you do not order an item for a long period of time. Assume again that you have stockpiled vacutainer needles and do not order for some time. During this time, you are actually using the needles daily from your stockpile, and the computer is reducing the medical supply level because there is no recorded consumption of the item. Finally, you use all your stockpile and submit an order to Medical Supply for more needles. However, the computer has reduced the stock level to zero, and Medical Supply has no needles.

If you extend these examples to each item in your section, the need to establish and adhere to realistic levels should be obvious. Always remember to order what you need, but be sure that you need what you order. If you order supplies once a week and use four boxes of needles each week, order four. The system works better that way.

Your requirement for an item may change. If it does, immediately advise Medical Supply personnel so that their level on the item can be adjusted accordingly. Medical Supply personnel cannot function effectively without your complete cooperation. They can give you only the quality of service you give them. It's a two-way street.

Regardless of your position, keep one thought in mind when working with Government supplies. Consider each supply item as being paid for by you "out of your pocket." If you adopt this attitude, maintaining good supply discipline will be a simple task.

Exercises (014):

1. What are two good reasons for using stock levels?

2. What maximum stock level are you authorized to maintain?

3. Upon what factor is the Medical Supply stock level based?

4. If you stockpile a particular supply item, how might this condition affect Medical Supply's stock of the item?

5. What action should you take if you want to change a stock level?

015. Cite procedures for budgeting for supplies and equipment.

Budgeting for Supplies and Equipment. Sooner or later you will become involved in budgeting for the supplies and equipment you use in your office. There is nothing difficult about budgeting, although it does require a certain amount of planning. We will consider the supply budget requirements first and then the equipment budget requirements. To both of these estimates, add 10 percent for inflation and price increases.

Supply budgeting. The resource management officer in your medical facility is responsible for preparation and submission of the annual budget for the entire facility. During December or January, he or she will request your department to furnish fund requirements for supplies for the coming fiscal year. The first action you should take when you receive this request is to study your previous supply expenditures. This is a simple matter, since your monthly issue lists show a total dollar amount on the last page of each list. You should determine how much you spent on supplies for the preceding fiscal year and how much you have spent in the first half of the current fiscal year. From this information you should be able to predict approximately how much you will need based on previous expenditures.

At this point, you should consider any upcoming changes in the workload and types of examination that might affect your requirements. For example, suppose you are to begin performing semiautomated or automated chemistry procedures during the coming fiscal year. Obviously you will need the reagents for your analyzers and other necessary devices to complete the system. Project these items into expenditures accordingly. Also, keep in mind other factors that may affect your workload, such as a forecasted increase or decrease in the base population.

Equipment budgeting. Equipment budgeting is also accomplished during December or January for the coming fiscal year. Some of the items you may need are a new semiautomated clinical chemistry system, a gamma counter, and a laboratory glassware washing machine.

Equipment budgeting is slightly different from supply budgeting in that you must have an authorization for the item. It must be authorized by the table of allowances (TA), a special document showing items of equipment authorized for medical facilities; or it must be authorized in writing from the major command. Usually, the request for an item requiring special authorization must be approved by a local Medical Equipment Review Board. Submit the request on AF Form 601.

Before you attempt to budget for a replacement item of equipment, make sure that you can fully justify your need for the item. Talk it over with your clinical laboratory officer and the medical equipment repair technicians. Factory alterations to existing equipment may solve your problem. Keep in mind that you must justify on AF Form 601 your need for any item; also, that equipment funds, as well as any other funds, are limited.

Exercises (015):

Indicate whether the following statements are true (T) or false (F). If you indicate "false," explain your answer.

_____ 1. Fiscal year budgets are usually prepared in December or January of the preceding fiscal year.

_____ 2. The resource management officer prepares and submits the annual budget for the entire medical facility.

_____ 3. When you are preparing your budget estimates, consider only your prior expenditures and new or replacement items of supplies and equipment.

_____ 4. You may find authorization for equipment items in the table of allowances.

_____ 5. You should submit requests for an equipment item on AF Form 601.

016. Define property responsibility and pecuniary liability, and state how you may be relieved from property responsibility.

Property Responsibility. Property responsibility is the obligation of Air Force members to care for Air Force property with which they are associated. The obligation to care for a particular property item is not limited to the individual who has signed for the item. It includes anyone who uses, supervises the use of, or otherwise comes in contact with the item. For example, the fact that you have not signed for the blood bank refrigerator in your department does not relieve you from the responsibility of properly caring for it. By "properly caring for property," we mean that you should take positive action to prevent the loss, damamge, or destruction of the equipment. Of course it is difficult to "lose" a blood bank refrigerator, but many smaller items of equipment can easily become lost.

Pecuniary liability. We in the Air Force also have "pecuniary liability" when it comes to caring for property. This means that an individual may have to pay for an item of equipment if the item is lost, damaged, or destroyed as a result of maladministration or negligence in the use, care, custody, or safekeeping of the item. In Air Force language, the "admission of pecuniary liability" by an individual implies that he or she is willing to pay the Air Force for the lost, damaged, or destroyed property.

Relief from property responsibility. You may be relieved of the responsibility for a particular piece of property in a number of ways. For example, you may turn the property in to Supply as excess, or you may transfer it to another person. However, if the property becomes destroyed, damaged, or lost, the procedure is not quite so simple. If you admit pecuniary liability, the least troublesome way to settle a monetary obligation is to pay in cash. The procedure is very simple. DD Form 1131, Cash Collection Voucher, is filled out, and you pay the Government in cash for the property. This collection method can be used only if the amount involved is less than $500.

If you admit pecuniary liability but do not have the money to pay in cash, DD Form 362, Statement of Charges for Government Property Lost, Damaged, or Destroyed, is used. The form simply authorizes the Government to deduct the amount from your paycheck. DD Form 362 is used only if the amount involved is less than $500.

Whenever a person does not admit pecuniary liability, or when the amount is $500 or more, an AF Form 198, Report of Survey for Air Force Property, must be prepared. Two commissioned officers are directly connected with the Report of Survey for Air Force Property. They are the appointing authority and the investigating officer. The appointing authority is a commander or other officer having jurisdiction over the individual who has custodial responsibility for the property in question. The appointing authority usually appoints an investigating officer, whose duty it is to make a detailed and impartial investigation (survey) of the circumstances connected with the loss, damage, or destruction of the property described on the Report of Survey for Air Force Property.

A survey officer is not necessarily appointed in every instance. When circumstances do not appear to warrant such a step, appointing authorities may make their own recommendations and forward the Report of Survey of Air Force Property to the base commander for review and approval. As a result of the findings, the person responsible for the custody of the property in question may or may not be required to pay for it.

Exercises (016):

1. What is property responsibility?

2. What responsibility do you have regarding all equipment in your laboratory, even though you may not have "signed" for it?

3. What is pecuniary liability?

4. If you admit pecuniary liability for an Air Force property item costing $490 that you lose, how may you settle your monetary obligation?

5. When must a Report of Survey of Air Force Property be prepared?

6. When a Report of Survey of Air Force Property is prepared, what action does the investigating officer take?

2-2. Safety in the Clinical Laboratory and in Clinical Chemistry

Safety, like a party guest, should be entertained, sustained, and maintained throughout the existence of the clinical laboratory. The awareness of safety will enable you to recognize the hazards in the clinical laboratory. Laboratory safety programs are plans required for the protection of all personnel, patients, and equipment. Because of many health, monetary, legal, and environmental involvements, such programs deserve attention by all laboratory workers. This section explains the significance of the Air Force Occupational Safety and Health (AFOSH) Program. This includes principles of laboratory safety, procedures for exercising safety precautions during job performance, and reporting accidents in the clinical laboratory.

017. Cite the purpose of the Air Force Occupational Safety and Health Program and some of the requirements that support the program.

Air Force Occupational Safety and Health Program. The Air Force conducts an intensive program to protect all Air Force personnel from work-related deaths, injuries, and occupational illness. It includes all safety, fire prevention, and health activities that affect the safety and health of Air Force personnel at their workplace.

The Air Force Occupational Safety and Health Policy Requirements. Development of a meaningful and effective safety program requires a concerted effort to identify the sources of hazards and the categories in which they belong. Thus, requirements must be followed by Air Force personnel in order to support the program.

The program requires that commanders provide all Air Force personnel a safe and healthful work environment in which recognized hazards have been eliminated or controlled. It requires that Air Force facilities, work areas such as hospital laboratories, and equipment comply with safety, fire, and health guidance. The program further requires that unsafe and unhealthy working conditions be eliminated or controlled through the use of engineering changes, administrative controls, or revised procedures. Qualified safety, fire prevention, and health personnel are required to inspect all workplaces for compliance with occupational safety and health requirements.

Air Force personnel are given the opportunity to participate in the AFOSH program without fear of coercion, discrimination, or reprisal. The names of persons identifying hazardous conditions will remain anonymous. Personnel are required to report and request inspections of unsafe or unhealthful working conditions to appropriate officials, such as supervisors, safety officers, or the bioenvironmental engineers.

Personnel should have access to OSHA and AFOSH standards and safety and health program procedures. Official time should be used by Air Force personnel to participate in AFOSH program activities.

The prime concern of the Air Force is to make safety a way of life for all personnel, to ultimately convince them of its benefits, and for personnel to realize that all safety violations can cause a dangerous and unnecessary risk of life and limb.

Exercises (017):

1. What is the purpose of the Air Force Occupational Safety and Health Program?

2. What does the Air Force require of commanders of the AFOSH program?

3. What does the Air Force require of hospital laboratories in support of its safety program?

4. The Air Force safety program requires that unsafe and unhealthy working conditions be eliminated or controlled through the use of what channels?

5. Reporting and inspection of unsafe or unhealthful working conditions may be made to whom?

018. Cite the purpose of the Air Force Occupational Safety and Health Standard and some general considerations for laboratory safety.

Air Force Occupational Safety and Health Standard. The basic purpose of the AFOSH Standard is to assist the managers of USAF medical organizations in maintaining a safe environment and to administer a safety program that conforms to Air Force directives. There are three general considerations for safety to the laboratory technicians. First, you are a highly trained and valuable resource; therefore, we do not want you to be "wasted" by a needless accident. Second, you can practice safety procedures for your own protection and that of your fellow workers. And third, you have the responsibility to (1) be aware of safety hazards, (2) follow policies and procedures designed to protect you, and (3) report all incidents or accidents so that steps may be taken to prevent recurrence.

Exercises (018):

1. Briefly state the purpose of the Air Force Occupational Safety and Health Standard.

2. Why is there serious concern for your safety in the laboratory?

3. When you practice safety procedures in the laboratory to whom is protection the prime concern?

4. What three guidelines, as your responsibility, should you follow in maintaining safety in the laboratory?

019. Select the appropriate guidelines for safety for medical laboratory technicians.

Requirements for Personal and Laboratory Safety. A general safety program must include orientation of new laboratory personnel to the department's attitudes and policies for assuring safe laboratory conduct. Please observe the following personal practices:

Smoking. Smoking is prohibited in the technical area. Smoke is annoying to some laboratory personnel, the burning cigarette is an ignition source to flammable solvents, and handling of cigarettes from bench to mouth is a means of exposure to both bacteria and certain toxic materials, such as mercury.

Eating and drinking. Eating and drinking are also prohibited in the technical work areas. It is in poor taste and unprofessional, a source of contamination, and specimens such as blood, urine, feces, and sputum containing a variety of pathogens are handled daily in the technical work area and stored in the laboratory refrigerators.

Food. Food is not permitted in technical refrigerators.

Application of cosmetics. Application of cosmetics in the technical work area is prohibited.

Contact lenses. Contact lenses will not be worn where eye hazards exist in the laboratory unless worn with approved goggles. Contact lenses, especially the soft ones, will absorb certain solvents and also constitute a hazard in splashes and spills. They offer no protection from a splash and may concentrate caustic material against the cornea or prevent tears from washing a caustic material away.

Some authorities feel that contact lenses should not be allowed in the laboratory even when safety goggles are worn because of the inability to remove the lenses and wash the eye in an emergency.

Face shields or eye protectors. Face shields or eye protectors must be worn when handling caustic materials.

Mouth pipetting. Mouth pipetting of specimens is prohibited. Pipetting aids are available for every task.

Hair. Hair must be secured back and off the shoulders in such a manner as to prevent it from coming into contact with contaminated materials or surfaces and also to prevent shedding of organisms into the work area. This is especially true in bacteriology.

Hand-to-face motions. Avoid hand-to-face motions when working with infectious or toxic materials.

Jewelry. Jewelry, such as watches and rings, will not be worn when handling infectious materials.

Clothing. Laboratory coats should be either worn in the lab and removed when out of the lab or worn over the uniform when out of the laboratory and removed when working. These policies may vary. The intent of the rule is to reduce the transfer of microorganisms from the lab to patients and vice versa.

Glassware. Glassware must be handled carefully to prevent injury. All broken, chipped, or cracked glassware must be immediately discarded in a specially marked separate container. Glass blowing and other artistic endeavors are prohibited. Disposal of broken glass along with paper and trash is a hazard to the custodial staff. Contaminated glassware must be decontaminated prior to discarding.

Exercises (019):

1. Match the subjects of personnel practices in column B with requirements in column A. Some column B items may be used more than once.

Column A	*Column B*
______(1) A source of ignition to flammable solvents; is a means of exposure to both bacteria and certain toxic material and is annoying to some laboratory personnel.	a. Smoking. b. Loosely groomed hair. c. Jewelry. d. Eating and drinking. e. Food. f. Mouth pipetting. g. Contact lenses.

Column A

_____(2) Is prohibited in the technical area.
_____(3) Is in poor taste and unprofessional and a source of contamination.
_____(4) Will absorb certain solvents and constitute a hazard in splashes or spills.
_____(5) Must be worn when handling caustic materials.
_____(6) Offer no protection from a splash and may concentrate material against the cornea or prevent tears from washing a caustic material away.
_____(7) Aids are available, so avoid this task.
_____(8) Shall be secured back and off the shoulders in such a manner as to prevent it from coming into contact with contaminated materials or surfaces.
_____(9) Should avoid such actions when working with infectious or toxic materials.
_____(10) Will not be worn when handling infectious or toxic materials.
_____(11) Should be either worn in the lab and removed when out of the lab or over the uniform when out of the laboratory and removed when working.
_____(12) Disposal of such items along with paper and trash is a hazard to the custodial staff.
_____(13) Must be decontaminated prior to discarding.
_____(14) Some authorities feel that they should not be allowed in the laboratory even when worn with safety glasses.

Column B

h. Clothing (lab coat).
i. Hand-to-face motions.
j. Face shields or eye protectors.
k. Glassware.
l. Application of cosmetics.

020. Cite the correct safety requirements and guidelines relating to procedures, reagents, and laboratory equipment.

Procedures, Reagents, and Equipment Safety. Each technician is responsible for following all safety guidelines as they relate to procedures, reagents, and equipment in the laboratory. Thus, maintaining safe working standards may require extra effort, but risk reduction must be a part of getting the job done correctly.

Hand washing. Hands must be washed frequently during work hours to remove any contaminating reagents or infectious materials. Hand washing or disinfection should be strictly enforced before leaving the laboratory, between patient contacts, and before eating or smoking.

Volatile flammables. Each refrigerator must be labeled on the outside of its door to denote whether or not it is safe for storage of flammables. If refrigeration is necessary for the storage of volatile solvents, these solvents are labeled as to their hazardous qualities and kept in explosion-proof refrigerators. The level of the solvents should be maintained in accordance with their daily use. Not more than 10 gallons or a 1-week's supply of flammable or combustible liquids will be maintained within the laboratory. Handle all flammable and toxic liquids in a space with good ventilation, preferably in a fume hood.

Compressed gas. Compressed gases used in the laboratory are a potential hazard. The most commonly used gases are oxygen with acetylene or methane, hydrogen or helium, nitrogen, propane, and carbon dioxide. The cylinders should be color coded, and the contents clearly marked. All cylinders must be securely fastened to the wall or bench or placed in floor holders so that they cannot overturn. If the cylinder falls, it can cause the outlet valve to rupture, and the cylinder may act like a torpedo and inflict serious injury.

When shutting down flammable gases, the gas is first turned off at the main take-in valve and the gas allowed to burn out; then the reduction valves are closed. Remember that propane is heavier than air and that a little leaking gas can flow along the top of the bench to be ignited by a flame elsewhere. Many flame photometers use small propane cylinders that are very convenient and involve the minimum difficulty.

Corrosives. Toxic corrosives such as mercury salts and/or caustic acids or alkalis must be handled with extreme caution. When handling these chemicals, you should wear goggles or a face mask.

Azides. Precautions should be taken with sodium azide, which is a common preservative in many in vitro diagnostic products. Sodium azide poured into drains reacts with metals in the plumbing and forms a powerful contact-sensitive explosive. Although the amount used as a preservative is relatively small, continued use with disposal through the sewer can result in a buildup of the metallic salts in the sewer pipes. It is best to avoid the use of these solutions containing azides particularly since they are also considered to be carcinogenic.

Carcinogenic hazards. Another hazard in the laboratory is the use of chemicals that are possibly carcinogenic. Among these are aromatic amines. An example is benzidine, which has been used frequently in the laboratory for testing hemoglobin, such as plasma hemoglobin or occult blood. A replacement by the compound 3,3', 5,5' tetramethylbenzene dihydrochloride is much safer.

Centrifuges. Centrifuges should be covered when operated. Many of the later models will not operate unless this is done. A common response among lab techs is to attempt to stop a slow spinning centrifuge. Do not attempt to stop or put anything in a spinning centrifuge, even if it is turning slowly. It is recommended that only centrifuges with safety interlocks be considered for new purchase. In addition, do not attempt to open the cover (in older centrifuges) until the rotor has completely stopped.

Do not centrifuge potentially infectious, volatile, or toxic substances in open tubes, as centrifugation may result in the formation of infectious aerosols or the volatilization of the liquid. If a tube breaks in the centrifuge, immediately turn the centrifuge off, and using rubber gloves, and if necessary, protective clothing, clean up. Any droplets in the chamber should be allowed to settle for about 15 minutes; then wash it with an appropriate solution, such as dilute acid for strongly alkaline solutions, sodium bicarbonate for acid solutions, and 5 percent sodium hypochlorite for potentially infectious material.

Caustic or corrosives. Caustic or corrosives are acids or alkalis which may cause burns of the skin, mouth, or eyes and may cause damage. Caution must be observed when using toxic or corrosive solutions. When handling these chemicals, the laboratory technician should wear goggles, or face mask, or work in a sink so that breaks or spills can be controlled. Wear aprons, gloves, and eye protection devices. Do not pipet by mouth. Do not sniff reagents. If considerable stirring is needed, a magnetic stirrer can be used to avoid splashing. In preparing reagents, use extreme care and add slowly. Always add ACID TO WATER (AWA) never water to acid. Allow acid to run down the side of the container and mix slowly by gentle rotation. Avoid overheating.

Exercises (020):

1. When should hand washing be done?

2. How are volatile solvents labeled and what type of refrigeration is used for their storage?

3. What is the maximum quantity of combustible liquid that may be maintained in the laboratory?

4. Where should all flammable and toxic liquids be handled?

5. Why is it important to prevent the compressed gas cylinder from falling?

6. If the No-Smoking rule is violated, what could result if a cigarette is left burning on the work counter within close proximity of a faulty or leaking propane cylinder?

7. Why should caution be observed in the use of reagents containing sodium azide as a preservative?

8. What solution can be used as a disinfectant in cleaning the chamber after the spillage or breakage involving infectious material?

9. When preparing reagents using a strong acid and water, in what order must they be added?

021. Cite first aid procedures in the chemistry laboratory.

First Aid Procedures. First aid procedures in the chemistry laboratory should be limited to those procedures that will prevent further loss or injury to body and preserve life before you can see a physician.

Chemical eye injuries. The medically recommended method of emergency treatment of chemical eye injuries is to wash the injured eye thoroughly with plain water for 15 minutes without delay! Remember that contact lenses prevent thorough irrigation and must be removed to prevent further injury by chemicals. A physician should be called at the first possible opportunity, preferably a specialist.

Chemical burns of the skin. When irritating chemicals come into contact with the skin or mucous membranes, injury begins instantly and first aid should be immediate. Chemicals such as acids, alkalis, or corrosive chemicals will cause burns the same as those caused by flame, steam, or hot liquid. If an acid or basic solution comes in contact with the skin, wash away the chemical completely as quickly as possible with copious quantities of running water until all traces of the chemical have been removed. No attempt should be made to neutralize the chemical until all areas of contact have been throughly irrigated with copious quantities of running water. For example, chemical burns caused by acetic acid may be neutralized with a mild alkaline solution of sodium bicarbonate. Five percent ammonium chloride or zinc chloride should be used to wash the affected areas promptly and throughly when chemical burns are caused by a caustic soda such as sodium hydroxide. However, if they are not immediately available, no time should be lost awaiting them, but copious amounts of water should be used. Remember, a physician should be called immediately after preliminary first aid is given. No oil or ointment of any kind should be applied to burned areas within the first 24 hours after contact or subsequently without the sanction of the attending physician.

Exercises (021):

1. What is the medically recommended method of emergency treatment of chemical eye injuries?

2. Why should contact lenses be removed from the eye during a chemical eye injury?

3. If an acid or basic solution should come in contact with the skin, what immediate action should you take?

4. What type of solution should be used to neutralize burns caused by acetic acid?

5. When chemical burns are caused by sodium hydroxide, what solutions may be used immediately on the affected area?

6. What kind of ointment should be applied to the burn within the first 24 hours?

022. Cite the reason and forms used for reporting laboratory accidents, incidents, and hazards.

Report of Accidents and Incidents. Every laboratory should have a standard procedure to follow in the event it becomes necessary to report an accident. Relatively minor incidents without personal injury or minor injury should be reported to your immediate supervisor. No group activity—whether children's games, professional sports, or a business financial progress—can intelligently advance without some form of scorekeeping. This is emphatically true in the laboratory safety surveillance program.

Detailed and accurate scorekeeping of accidents not only permits the laboratory supervisor to see where his or her department has been; it allows that person to look ahead. A record can point out accident trends, high hazard areas, and frequency and severity of incidents involving personnel and equipment. Another benefit of proper recordkeeping is protection against false claims by unscrupulous individuals, whether they be lab techs, visitors, or patients.

As a 7-level supervisor, you should investigate, document, and report all accidents. The diagnosis, treatment, and prognosis is made easier when records from well-documented incidents are available. This also makes the analysis of health records for future disability claims easier to interpret and evaluate. Accident reporting protects you, so don't neglect it. Do not let your co-workers forget it either. You are in a career field where occupational diseases are as numerous as pathogenic bacteria.

Reportable Accidents. Every accident—whether it involves an injury or not—should be reported. Accidents resulting in damage to laboratory instruments and equipment and the building should be reported. All fatalities, injuries requiring hospitalization, injuries involving three or more people, property damage, biologic exposure resulting in lost time or which may involve the public, and accidents resulting in spillage of radioactive materials should be reported on AF Form 765, Hospital Incident Statement. This form may also be used to report situations that may result in an accident.

All situations or conditions that have potential for injury, hazard to health, or damage to laboratory equipment should be reported on AF Form 457, USAF Hazard Report. You may obtain assistance from your unit safety representative in completing this form. Remember that you as the supervisor must document accurate detailed information of all accidents, incidents, and hazards. Such information will be required by the hospital risk manager and become a vital record to be helpful in future investigation of the same or future situations of similar characteristics.

Exercises (022):

1. What are some advantages of keeping records of accidents in the laboratory?

2. What are some advantages of well-documented reports of accidents?

3. What form should you use to report accidents in the laboratory?

4. What form(s) should you use to report a situation that could result in any accident?

General Medical Laboratory Administration

YOUR KEY SUPERVISORY duty, aside from orienting newly assigned personnel, evaluating their performance, scheduling work assignments, and preparing position descriptions, is to monitor the efficient operation of the clinical laboratory administration. The keystone of the clinical laboratory is an effectively organized laboratory where guidelines and policies are available for all concerned personnel to follow. You can provide the leadership for such a laboratory. This chapter is designed to help you make the transition from the duties of a specialist to those of a technician with increased supervisory knowledge and responsibilities.

3-1. Standard Operating Procedures

Consider modern administrative techniques and you immediately think of paperwork. This is true whether you think of medical laboratory administration or running your home. If you think of personal and family life for a moment, you will realize at once that its orderly existence depends upon carefully prepared but usually lightly regarded pieces of paper. The bank balance sheet, driver's license, marriage certificate, property abstract, and personal and business letters are only a few examples of paperwork essential for the everyday living of modern men and women. Just as these things bring order to everyday life, administrative functions make order possible in the medical laboratory.

023. Cite administrative policies for the operation of the clinical laboratory in terms of standard operating procedures and standard forms.

Contents of Standard Operating Procedures. Every laboratory should have a booklet, form, or guide to laboratory services that lists all the specific examinations and services that the laboratory can normally provide. This information should be compiled by the laboratory officer, superintendent, NCOIC, or pathologist and coordinated with other hospital services.

Upon approval and signature of the hospital commander, it can be developed into a standard operating procedure. A copy should be make available to each assigned physician, dentist, nurse, unit or clinic supervisor, and member of the laboratory staff. The booklet should include the following information:

a. Emergency procedures.
b. Routine procedures.
c. Tests beyond the capability of the laboratory which require transfer to a reference laboratory for completion.
d. The method and normal range for each procedure normally performed.
e. Direction for the preparation of the patient.
f. The type of specimen, the amount of specimen, and the type of anticoagulant or preservative, if required.
g. Tests and procedures which may be limited to a certain day or days for completion.
h. Policies relating to blood bank activities.
i. Policies relating to the pathology section.
j. Schedules stating the expected length of time required to complete certain types of procedures.
k. Methods for reporting test results to the requesting physicians.
l. Any other information desired.

Standard Forms. Physicians use the standard forms to make routine medical laboratory requests. You should maintain a supply of these forms for the laboratory office in case a unit or clinic runs short. Table 3-1 lists most of the standard forms. Requisitioning and reporting has been standardized through the use of standard forms.

Standard forms are signed by the physicians or dentists for the tests and procedures that they desire unless they personally signed AF Form 3066, Doctor's Orders, or have given verbal approval in case of an emergency. In the latter case, a nurse, a senior medical service technician, or similar individual assigned to the staff may prepare the forms for them.

After completing the test, you enter the test results on the standard form and insert your initials or signature, whichever is the procedure in your laboratory. Completed forms are usually signed by the person in charge of the laboratory or authorized representative. The standard forms are prepared in triplicate. The first copy is sent to the unit or clinic for the patient's records, the second copy is the doctor's copy, and the third or hard copy is filed in the laboratory. Computerized laboratories may file a composite daily master computer log instead of individual laboratory reports.

Completed copies of Standard Forms 550, Urinalysis, and 553, Microbiology I, are shown in figure 3-1. Figure 3-2 represents a completed Standard Form 518, Medical record—Blood or Blood Component Transfusion. These forms are maintained in a separate file series from the other standard forms used for routine laboratory procedures.

Those facilities having a Coulter Counter Model S use AF Form 1976, Hematology, for appropriate requests

TABLE 3-1

STANDARD FORMS USED BY THE MEDICAL LABORATORY

Standard Form Number	Title
546	Chemistry I
547	Chemistry II
548	Chemistry III (Urine)
549	Hematology
550	Urinalysis
551	Serology
552	Parasitology
553	Microbiology I
554	Microbiilogy II
555	Spinal Fluid
556	Immunohematology
557	Miscellaneous
518	Medical Record--Blood or Blood Component Transfusion

instead of SF 549, Hematology. Certain other types of automated laboratory equipment with printout capabilities may require special request forms. These forms are maintained in a separate file series from the other standard form used for routine laboratory procedures.

Within each category, file the forms alphabetically by the patient's last name. You can use a category breakdown as follows: chemistry, microbiology, urinalysis, hematology, immunohematology, and miscellaneous. Headings for the categories are a matter of choice. Their function is for convenience in locating a file copy. Tests that do not fit exactly into one of the chosen category headings may be filed in the one to which it is most closely associated. For example, SF 555, Spinal Fluid, may be placed in the category established for hematology.

When more than one test or procedure has been performed for the same patient, file the most recent report in front of the forms from previous dates. The important point is to make sure that each member of the laboratory staff is familiar with the filing system.

These standard forms must be retained in the file for a minimum of 12 months. Figure 3-3 shows one method for establishing a filing system. Dispose of files as directed by AFR 12–50, *Disposition of Air Force Documentation*. This will be discussed in another section.

Exercises (023):

1. What persons are responsible for compiling the information in the clinical laboratory guide?

2. What information should be included in the laboratory guide?

3. How are the triplicate copies of standard laboratory forms routed?

4. Instead of individual laboratory reports, how may they be filed in computerized laboratories?

5. Reports on standard laboratory forms are filed in what manner?

6. How long are the standard forms used for routine laboratory tests retained in the file?

3-2. Publications

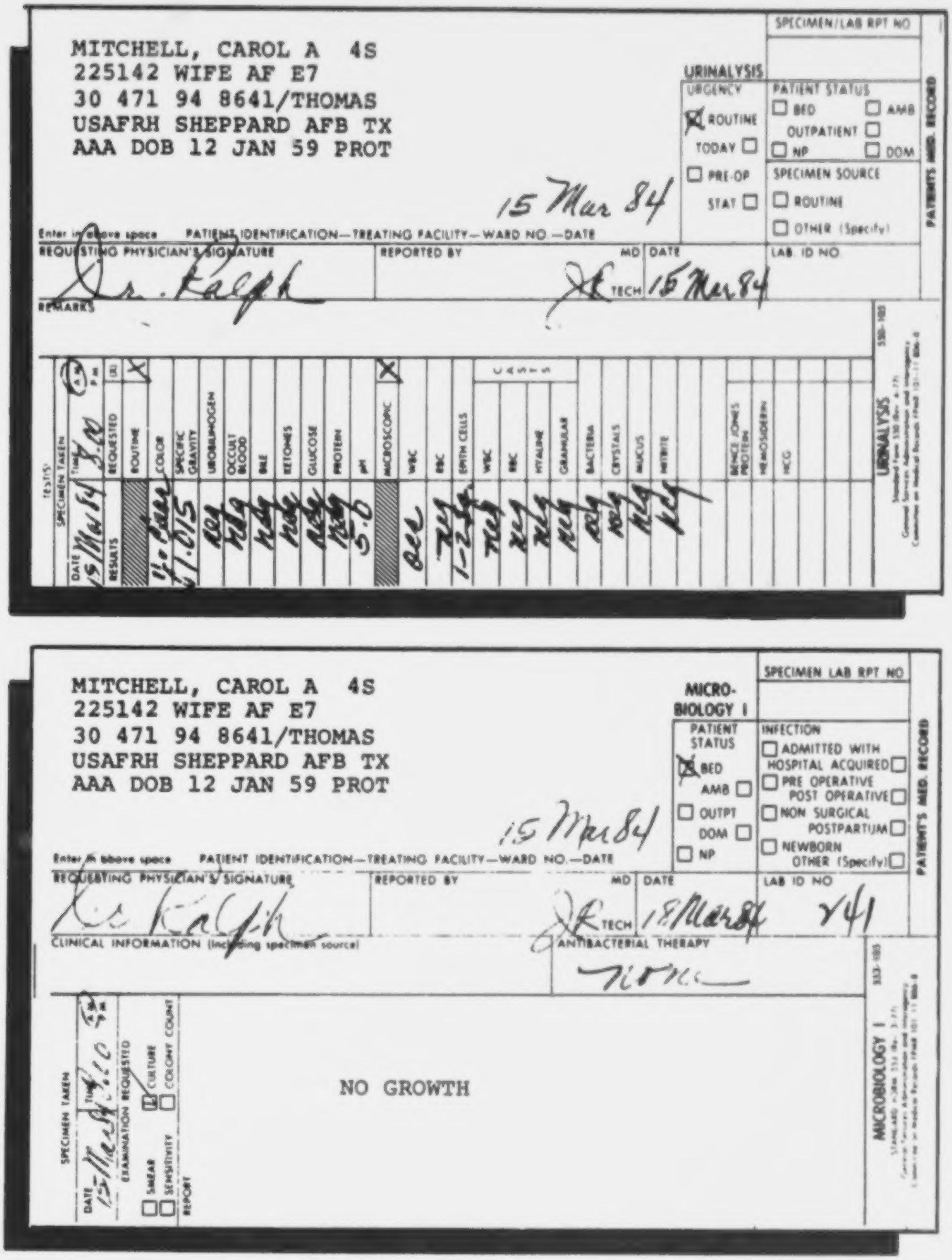
Every clinical laboratory must have some means of communicating its policy and guidelines from the managers to the workers. Verbal communication may be the quickest, but most people like to see important news "on paper" to avoid the possibility of misinterpretation. The Air Force communicates important news and policies via written documents called publications. Since some of those publications affect your duties in the clinical laboratory, you should be able to find and use pertinent publications in order to maintain uniformity of service and standardization of procedures.

024. Identify the two general classes of Air Force Publications, the purpose of AFR 0–2, and show how it is used to locate a publication.

Classes of Air Force Publications. Air Force publications are divided into two general classes: departmental and field.

Departmental publications. Departmental publications are issued by, or for, Hq USAF and one or more major air commands or by Hq USAF alone. An example of a departmental publication would be AFR 168–4, *Administration of Medical Activities*.

Field publications. Field publications originate at major air command level or below and are used only within the issuing activity. An example of a field publication is ATCR 160–25, *Medical Readiness Planning*.



MITCHELL, CAROL A 4S
225142 WIFE AF E7
30 471 94 8641/THOMAS
USAFRH SHEPPARD AFB TX
AAA DOB 12 JAN 59 PROT

15 Mar 84

Enter in above space PATIENT IDENTIFICATION—TREATING FACILITY—WARD NO.—DATE

URINALYSIS

SPECIMEN/LAB RPT NO

URGENCY: ☒ ROUTINE ☐ TODAY ☐ PRE-OP ☐ STAT

PATIENT STATUS: ☐ BED ☐ AMB ☐ OUTPATIENT ☐ NP ☐ DOM

SPECIMEN SOURCE: ☐ ROUTINE ☐ OTHER (Specify)

REQUESTING PHYSICIAN'S SIGNATURE: Dr. Ralph

REPORTED BY: MD TECH

DATE: 15 Mar 84

LAB. ID NO.

REMARKS

SPECIMEN TAKEN DATE	15 Mar 84
TIME	8:00 [✓ A.M.] P.M.
ROUTINE	X
COLOR	[illegible] Clear
SPECIFIC GRAVITY	1.015
UROBILINOGEN	neg
OCCULT BLOOD	neg
BILE	neg
KETONES	neg
GLUCOSE	neg
PROTEIN	neg
pH	5.0
MICROSCOPIC	X
WBC	occ
RBC	neg
EPITH CELLS	1-2 hpf
CASTS WBC	neg
CASTS RBC	neg
CASTS HYALINE	neg
CASTS GRANULAR	neg
BACTERIA	neg
CRYSTALS	neg
MUCUS	neg
NITRITE	neg
BENCE JONES PROTEIN	
HEMOSIDERIN	
HCG	

PATIENT'S MED. RECORD

550-103

URINALYSIS

MITCHELL, CAROL A 4S
225142 WIFE AF E7
30 471 94 8641/THOMAS
USAFRH SHEPPARD AFB TX
AAA DOB 12 JAN 59 PROT

15 Mar 84

Enter in above space PATIENT IDENTIFICATION—TREATING FACILITY—WARD NO.—DATE

MICROBIOLOGY I

SPECIMEN LAB RPT NO

PATIENT STATUS: ☒ BED ☐ AMB ☐ OUTPT ☐ DOM ☐ NP

INFECTION: ☐ ADMITTED WITH ☐ HOSPITAL ACQUIRED ☐ PRE OPERATIVE ☐ POST OPERATIVE ☐ NON SURGICAL ☐ POSTPARTUM ☐ NEWBORN ☐ OTHER (Specify)

REQUESTING PHYSICIAN'S SIGNATURE: Dr Ralph

REPORTED BY: MD TECH

DATE: 18 Mar 84

LAB ID NO: 741

CLINICAL INFORMATION (including specimen source)

ANTIBACTERIAL THERAPY: None

SPECIMEN TAKEN DATE: 15 Mar 84 TIME: 8:00 A.M.

EXAMINATION REQUESTED: ☐ SMEAR ☐ SENSITIVITY ☒ CULTURE ☐ COLONY COUNT

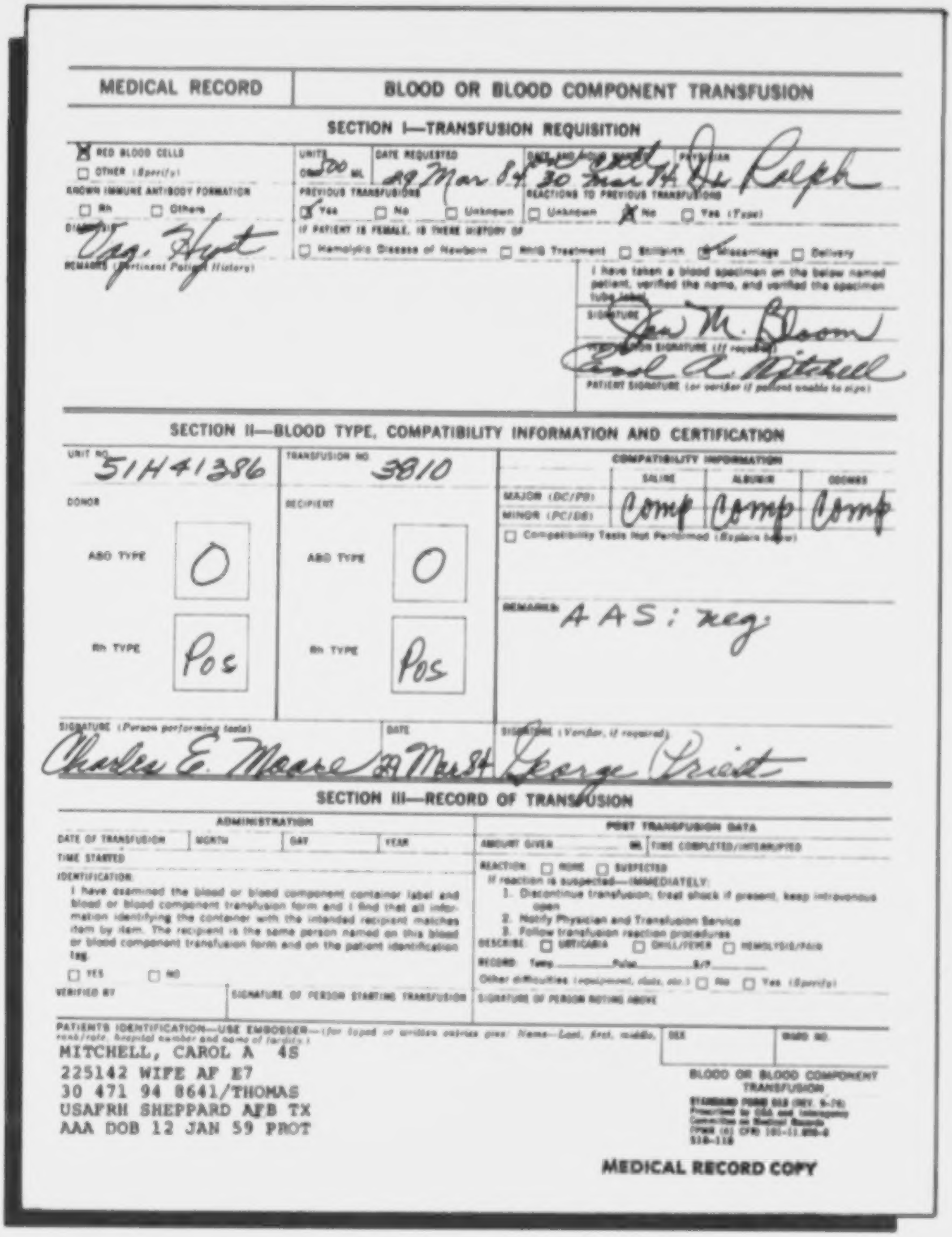
REPORT: NO GROWTH

PATIENT'S MED. RECORD

553-103

MICROBIOLOGY I

Figure 3–1. Sample of Standard Forms 550 and 553.



MEDICAL RECORD | **BLOOD OR BLOOD COMPONENT TRANSFUSION**

SECTION I—TRANSFUSION REQUISITION

☒ RED BLOOD CELLS
☐ OTHER (Specify)

UNITS OR 300 ML

DATE REQUESTED 29 Mar 84

DATE AND HOUR WANTED 30 Mar 84

PHYSICIAN Dr Ralph

KNOWN IMMUNE ANTIBODY FORMATION ☐ Rh ☐ Others

PREVIOUS TRANSFUSIONS ☑ Yes ☐ No ☐ Unknown

REACTIONS TO PREVIOUS TRANSFUSIONS ☐ Unknown ☒ No ☐ Yes (Type)

DIAGNOSIS Vag. Hyst

IF PATIENT IS FEMALE, IS THERE HISTORY OF
☐ Hemolytic Disease of Newborn ☐ RhIG Treatment ☐ Stillbirth ☑ Miscarriage ☐ Delivery

REMARKS (Pertinent Patient History)

I have taken a blood specimen on the below named patient, verified the name, and verified the specimen tube label.

SIGNATURE Jan M. Bloom

VERIFICATION SIGNATURE (if required)

Carol A. Mitchell

PATIENT SIGNATURE (or verifier if patient unable to sign)

SECTION II—BLOOD TYPE, COMPATIBILITY INFORMATION AND CERTIFICATION

UNIT NO. 51H41386

TRANSFUSION NO. 3810

	DONOR	RECIPIENT
ABO TYPE	O	O
Rh TYPE	Pos	Pos

COMPATIBILITY INFORMATION

	SALINE	ALBUMIN	COOMBS
MAJOR (DC/PS) MINOR (PC/DS)	Comp	Comp	Comp

☐ Compatibility Tests Not Performed (Explain below)

REMARKS AAS: neg

SIGNATURE (Person performing tests) Charles E. Moore

DATE 29 Mar 84

SIGNATURE (Verifier, if required) George Priest

SECTION III—RECORD OF TRANSFUSION

ADMINISTRATION

DATE OF TRANSFUSION | MONTH | DAY | YEAR

TIME STARTED

IDENTIFICATION

I have examined the blood or blood component container label and blood or blood component transfusion form and I find that all information identifying the container with the intended recipient matches item by item. The recipient is the same person named on this blood or blood component transfusion form and on the patient identification tag.

☐ YES ☐ NO

VERIFIED BY | SIGNATURE OF PERSON STARTING TRANSFUSION

POST TRANSFUSION DATA

AMOUNT GIVEN ML | TIME COMPLETED/INTERRUPTED

REACTION ☐ NONE ☐ SUSPECTED

If reaction is suspected—IMMEDIATELY:

1. Discontinue transfusion; treat shock if present; keep intravenous open
2. Notify Physician and Transfusion Service
3. Follow transfusion reaction procedures

DESCRIBE: ☐ URTICARIA ☐ CHILL/FEVER ☐ HEMOLYSIS/PAIN

RECORD: Temp. _____ Pulse _____ B/P _____

Other difficulties (equipment, clots, etc.) ☐ No ☐ Yes (Specify)

SIGNATURE OF PERSON NOTING ABOVE

PATIENTS IDENTIFICATION—USE EMBOSSER—(for typed or written entries give: Name—Last, first, middle; rank/rate, hospital number and name of facility) | SEX | WARD NO.

MITCHELL, CAROL A 4S
225142 WIFE AF E7
30 471 94 8641/THOMAS
USAFRH SHEPPARD AFB TX
AAA DOB 12 JAN 59 PROT

BLOOD OR BLOOD COMPONENT TRANSFUSION
STANDARD FORM 518 (REV. 9-78)
Prescribed by GSA and Interagency Committee on Medical Records
FPMR (41 CFR) 101-11.806-8
518-118

MEDICAL RECORD COPY

Figure 3-2. Sample of Standard Form 518.

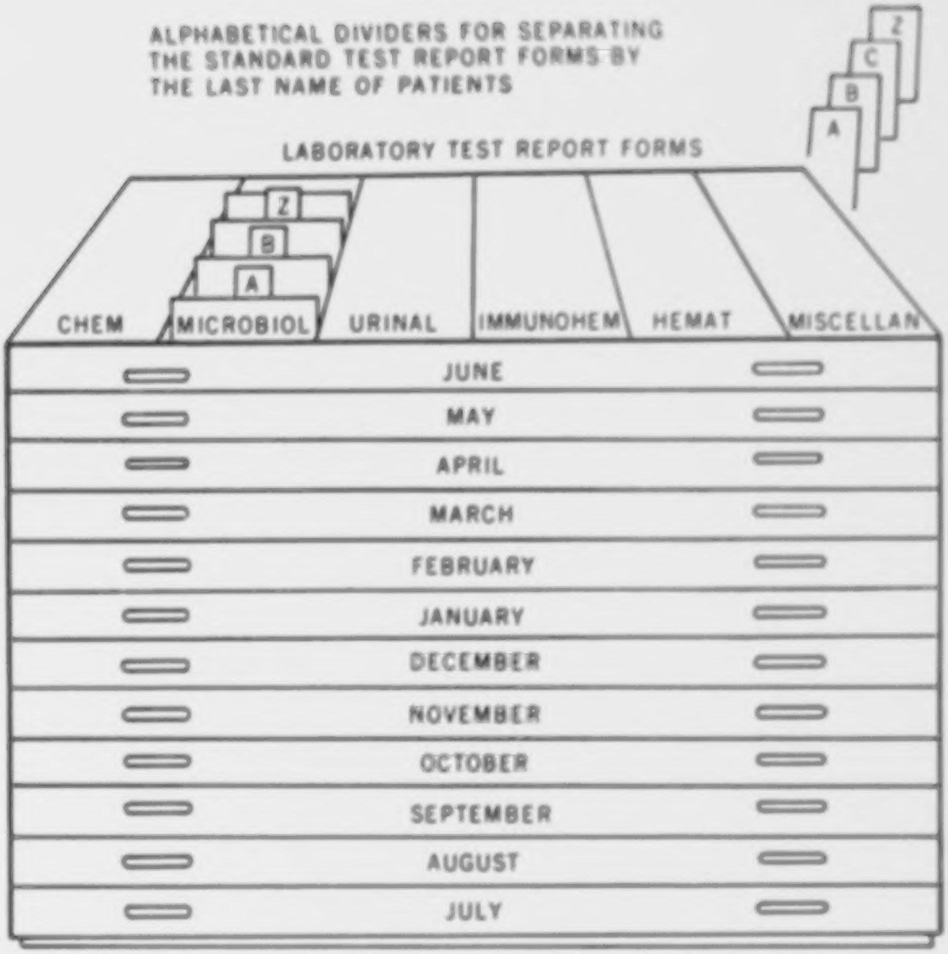

ALPHABETICAL DIVIDERS FOR SEPARATING THE STANDARD TEST REPORT FORMS BY THE LAST NAME OF PATIENTS
LABORATORY TEST REPORT FORMS
Z
C
B
A
CHEM
MICROBIOL
URINAL
IMMUNOHEM
HEMAT
MISCELLAN
JUNE
MAY
APRIL
MARCH
FEBRUARY
JANUARY
DECEMBER
NOVEMBER
OCTOBER
SEPTEMBER
AUGUST
JULY

Figure 3-3. One method for establishing a file system.

Locating Publications Using AFR 0-2, ***Numerical Index of Standard and Recurring Air Force Publications.*** Of the many departmental indexes of publications, AFR 0-2 gives the most information about each publication and will be your most frequently used index. The 0-2 provides an alphabetical listing of the basic subject series and a numerical listing of each publication subject and title.

The numerical listing of AFR 0-2 is arranged by publication series. The series number is a reference to the subject of the publications. For example, all publications dealing with medical administration are numbered 168 and are referred to as the 168 series. Other medical series are shown in table 3-2.

In addition to the series number, each publication has a control number, preceded by a dash (for example, 168-1, 168-2, etc.). Control numbers are normally assigned in sequence beginning with number 1 for each series. Figure 3-4 further amplifies the publications numbering system. From figure 3-4, we can tell a great deal about AFR 5-4. First, we know that it is a departmental publication because of the "AF" in AFR. Second, we know that the type of publication is a regulation because of the "R."

If our example in figure 3-4 had been TACR 5-4, then we would have known that it was a field publication and that in order to find out its title, we would have to check TAC Regulation 0-2, which is the numerical index for Tactical Air Command publications. Because one publication cannot duplicate information contained in another publication, TACR 5-4 would not be the same as AFR 5-4. However, if TAC would like to clarify or add to AFR 5-4, it could issue a supplement to this publication. Supplements are always issued at a command level which is subordinate to the echelon of command that published the original. Publication changes, however, are issued at the same echelon of command that issued the original.

As an example of how the AFR 0-2 format is use to locate a publication let's suppose that you were interested in finding out if there were any type of AF publication on blood bank standards. You would first look in AFR 0–2, *Numerical Index of Standard and Recurring Air Force Publications*, page 2. This index is listed alphabetically by subject and numerical series. You would look under the general heading "Medical Service," and note that it is the heading for the 160 series. Turn to the 160 series in the 0–2 and proceed to look for the subject, noting the titles listed. "Standards for Blood Banks and Transfusion Services" is listed opposite AFR 160–24. This means that information on blood bank standards can be found in AFR 160–24.

Exercises (024):

1. What are the two classes of Air Force publications?

2. Which of the two classes of Air Force publications normally originates at major air commands and below?

3. What is the purpose of AFR 0-2?

4. If you had to obtain an Air Force publication on The Aerospace Medicine Program, to what numerical series would you refer in AFR 0–2?

5. What type of publication is ATCR 160–26? (Field or Departmental?)

TABLE 3-2
USAF MEDICAL PUBLICATIONS SERIES NUMBERS

SERIES NO.	NAME
160-	Medical Service
161-	Aerospace Medicine
162-	Dental Services
163-	Veterinary Service
164-	Aeromedical Evacuation
167-	Medical Materiel
168-	Medical Administration
169-	Medical Education and Research

6. If you used the AFR 0–2, in what series would you locate Air Force regulations pertaining to Medical Education and Research?

025. Cite procedures for requesting Air Force and civilian publications.

How to Request Publications. Now that you know what publications are and how they are numbered and listed in AFR 0–2, how do you get them?

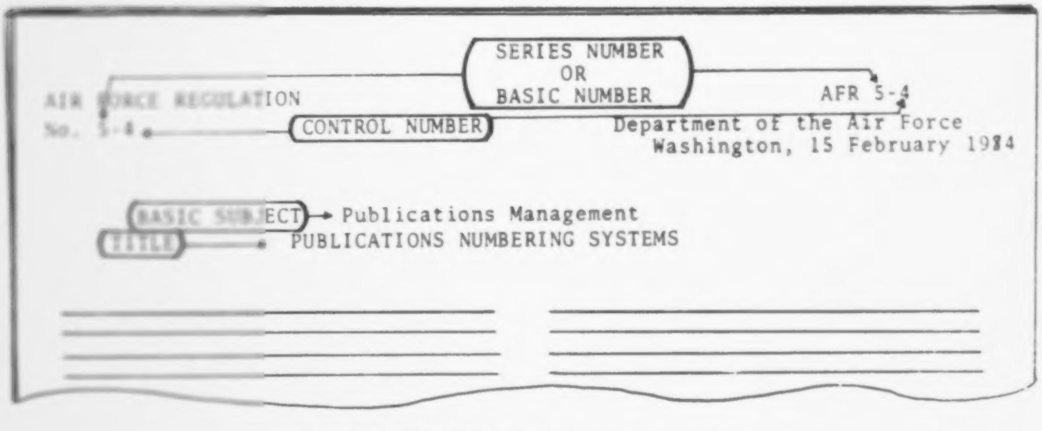

SERIES NUMBER OR BASIC NUMBER
AIR FORCE REGULATION
No. 5-4
CONTROL NUMBER
AFR 5-4
Department of the Air Force
Washington, 15 February 1984
BASIC SUBJECT
Publications Management
TITLE
PUBLICATIONS NUMBERING SYSTEMS

Figure 3–4. The USAF publications numbering system.

Air Force publications. The chief of personnel and administrative services is responsible for supervising all official publications used in your medical facility. Each section within the medical facility must establish its publications requirements with the office of Personnel and Administrative Services. If you want to obtain a publication for the laboratory, you must send a memo to this office listing the publication number, title, justification, and the number of publications required. The office of Personnel and Administrative Services will review the list for essentiality and then consolidate the requirements from all sections. This office then orders each basic publication (including its changes to date) and prepares a separate request to have your hospital included on the series distribution list at the publications distribution office. This assures that you will be included when new changes to your publication come out.

Civilian publications. When there is a need for a civilian publication—for example, a medical reference book or visual science dictionary—you should do one of the following:

a. If your hospital has a medical library, you should submit a request to the librarian. Although the exact format of your request may vary according to local policy generally the librarian will need the following information:

(1) Stock number (if applicable).
(2) Description (title, edition, author, and publisher).
(3) Unit cost.
(4) Number required.
(5) Justification (in narrative form—short paragraphs).

b. If your hospital does not have a library, civilian publications are usually ordered through Medical Materiel. Check your local policy for the exact procedure.

Exercises (025):

1. If you want to obtain an Air Force publication, to what office should your memo be sent and what information must be included?

2. After your request for publication has been accepted, it will be included on the series distribution list at what office?

3. If you want to obtain a civilian publication, dependent upon your local policy, to what offices should you submit your request?

3-3. File and Records

Just how do you keep track of all the forms, records, letters, regulations, order blanks, catalogs, logbooks, etc., used in your laboratory? Do you simply dump everything in your bottom left desk drawer and wade through a bunch of loose papers every time you have to look up something, or do you maintain an orderly filing system that can be understood and used by everyone in the laboratory? The Air Force has suggestions for maintaining an orderly documentation system, and the essence of these suggestions is printed here.

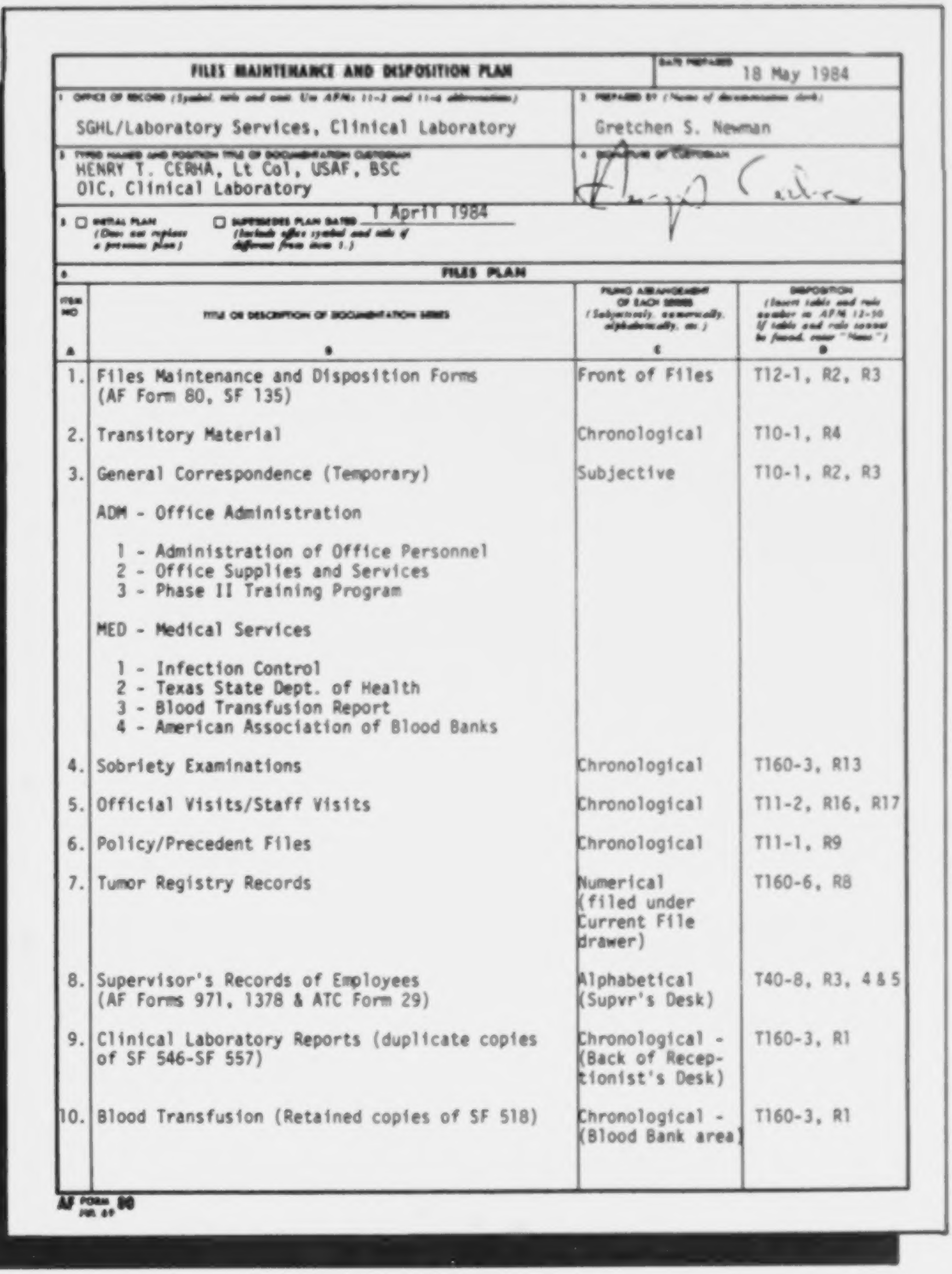
026. Identify the directives that are concerned with procedures and techniques for maintenance and disposition of current documentation, specify the filing sequence and manner of disposition, and name the forms used for such documentation.

Categories of Documentation. The Air Force categorizes documentation as official, private, or personal. All material that constitutes documentation is defined in AFR 12-1, *Air Force Documentation Management Program*, and copies of this material made by an Air Force activity are *official* documentation. Organized collections of official documentation are official files. Official files that are used frequently in conducting current business are maintained in the custody of offices responsible for that function. These offices are known as offices of record, according to AFR 12–20, *Management of Documentation*. AFR 12–20 also gives the policies, procedures, and techniques for maintaining documentation files.

Disposition Policies. AFR 12–50, *Disposition of Air Force Documentation*, Chapter 4, outlines the disposition policies and procedures applicable to Air Force documentation. No Air Force documentation may be destroyed unless it is authorized in the disposition standards in AFR 12–50.

Medical Laboratory Files. Official documentation held by the medical laboratory, the office of record, may include some or all of the titles shown in figure 3-5A and B. If your laboratory maintains official files of any subjects described, you must include in the file an AF Form 80, Files Maintenance and Disposition Plan, and an AF Form 82, Files Disposition Control Label, to identify the documentation and to prescribe its retirement or destruction. Prepare these forms in the manner described below.

AF Form 80, Files Maintenance and Disposition Plan. Each office of record should prepare an AF Form 80 (fig. 3-5A and B) to identify all documentation for which the office has responsibility. As a minimum, the AF Form 80 should be prepared in duplicate as directed in local supplements to AFR 12–20. The office of record keeps the original in front of the current files. The duplicate is forwarded to the documentation manager. You should review and amend AF Form 80 periodically to insure that all files and documents are accounted for, and that cited disposition authorities are appropriate. Notify the documentation manager of any changes. Use the same form



FILES MAINTENANCE AND DISPOSITION PLAN

DATE PREPARED: 18 May 1984

1. OFFICE OF RECORD (Symbol, title and unit. Use AFMs 11-2 and 11-4 abbreviations): SGHL/Laboratory Services, Clinical Laboratory

2. PREPARED BY (Name of documentation clerk): Gretchen S. Newman

3. TYPED NAME AND POSITION TITLE OF DOCUMENTATION CUSTODIAN:
HENRY T. CERHA, Lt Col, USAF, BSC
OIC, Clinical Laboratory

4. SIGNATURE OF CUSTODIAN

5. ☐ INITIAL PLAN (Does not replace a previous plan) ☐ SUPERSEDES PLAN DATED 1 April 1984 (Include office symbol and title if different from item 1.)

6. FILES PLAN

ITEM NO A	TITLE OR DESCRIPTION OF DOCUMENTATION SERIES B	FILING ARRANGEMENT OF EACH SERIES (Subjectively, numerically, alphabetically, etc.) C	DISPOSITION (Insert table and rule number in AFM 12-50. If table and rule cannot be found, enter "None.") D
1.	Files Maintenance and Disposition Forms (AF Form 80, SF 135)	Front of Files	T12-1, R2, R3
2.	Transitory Material	Chronological	T10-1, R4
3.	General Correspondence (Temporary)	Subjective	T10-1, R2, R3
	ADM - Office Administration		
	1 - Administration of Office Personnel 2 - Office Supplies and Services 3 - Phase II Training Program		
	MED - Medical Services		
	1 - Infection Control 2 - Texas State Dept. of Health 3 - Blood Transfusion Report 4 - American Association of Blood Banks		
4.	Sobriety Examinations	Chronological	T160-3, R13
5.	Official Visits/Staff Visits	Chronological	T11-2, R16, R17
6.	Policy/Precedent Files	Chronological	T11-1, R9
7.	Tumor Registry Records	Numerical (filed under Current File drawer)	T160-6, R8
8.	Supervisor's Records of Employees (AF Forms 971, 1378 & ATC Form 29)	Alphabetical (Supvr's Desk)	T40-8, R3, 4 & 5
9.	Clinical Laboratory Reports (duplicate copies of SF 546-SF 557)	Chronological - (Back of Receptionist's Desk)	T160-3, R1
10.	Blood Transfusion (Retained copies of SF 518)	Chronological - (Blood Bank area)	T160-3, R1

AF FORM 80 JAN 69

Figure 3-5A. Sample of AF Form 80 (front).

for changes; just delete noncurrent information and enter new information.

AF Form 82, Files Disposition Control Label. After you have prepared an AF Form 80 (fig. 3-5A and B), prepare an AF Form 82 (fig. 3-6) for each subitem with a different retention period. AF Form 82 is printed on a paper stock with an adhesive back. Affix the AF Form 82 to the tabs of the guide cards in front of each documentation series, or in a conspicuous location on the container housing the documentation. If the material is kept in several containers, AF Form 82 may be affixed to the first container or to each container. AFR 12-20 contains a listing of the types of filing equipment authorized for storing official documentation. (Basically, you can use a letter-size file cabinet, or a file box, but not your desk unless the volume of files is small.)

Exercises (026):

1. What basic directive is concerned with policies, procedures, and techniques for maintaining documentation files?

2. What directive gives disposition policies and procedures for AF documentation?

3. Using the Files Maintenance and Disposition Plan (fig. 3-5A and B), indicate the filing arrangement in which Blood Transfusion Report Records are filed.

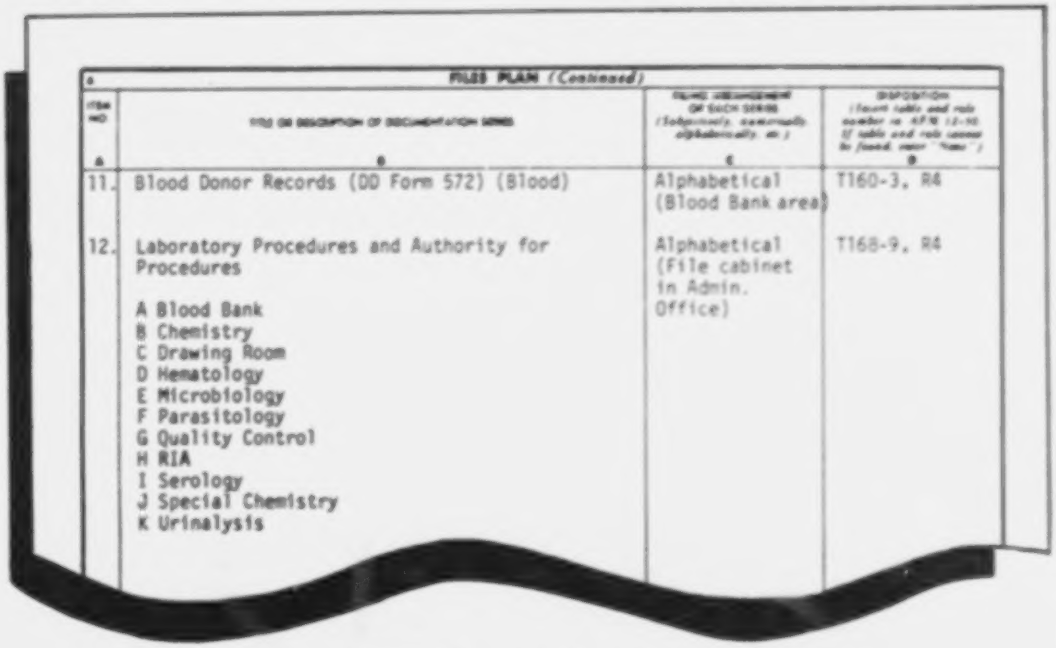
4. What rule number in AFR 12-50 determines the manner of disposition for the records in exercise 3?

5. What form must be completed showing all the official documentation held in your laboratory?

6. What form must be affixed to each folder or container of official documentation?

3-4. Ethics and Interpersonal Relations in the Medical Laboratory

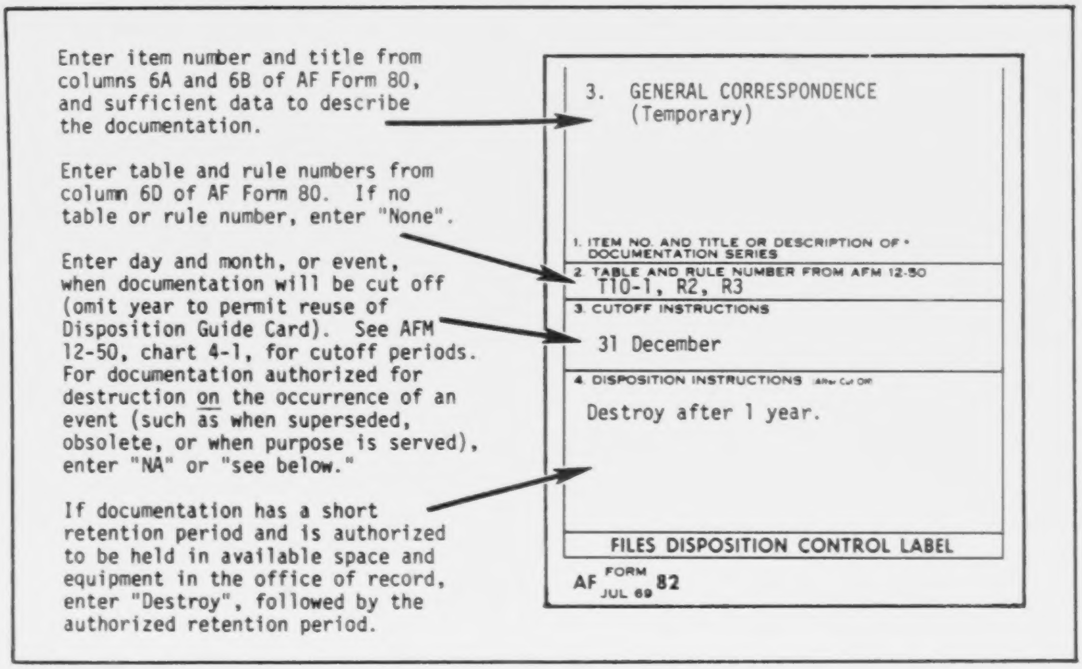
What would you do if you worked in a laboratory suspected of turning out false reports? Or doing poor work? In addition, the techs don't get "along" with each other, or with physicians and other personnel in the hospital. Patients are frequently dissatisfied with the service and would rather be treated elsewhere because they distrust your laboratory service. What would you do?



FILES PLAN (Continued)

ITEM NO. a	TITLE OR DESCRIPTION OF DOCUMENTATION SERIES b	FILING ARRANGEMENT OF EACH SERIES c	DISPOSITION d
11.	Blood Donor Records (DD Form 572) (Blood)	Alphabetical (Blood Bank area)	T160-3, R4
12.	Laboratory Procedures and Authority for Procedures A Blood Bank B Chemistry C Drawing Room D Hematology E Microbiology F Parasitology G Quality Control H RIA I Serology J Special Chemistry K Urinalysis	Alphabetical (File cabinet in Admin. Office)	T168-9, R4

Figure 3-5B. Sample of AF Form 80 (back).



Enter item number and title from columns 6A and 6B of AF Form 80, and sufficient data to describe the documentation.

Enter table and rule numbers from column 6D of AF Form 80. If no table or rule number, enter "None".

Enter day and month, or event, when documentation will be cut off (omit year to permit reuse of Disposition Guide Card). See AFM 12-50, chart 4-1, for cutoff periods. For documentation authorized for destruction on the occurrence of an event (such as when superseded, obsolete, or when purpose is served), enter "NA" or "see below."

If documentation has a short retention period and is authorized to be held in available space and equipment in the office of record, enter "Destroy", followed by the authorized retention period.

3. GENERAL CORRESPONDENCE
(Temporary)

1. ITEM NO. AND TITLE OR DESCRIPTION OF DOCUMENTATION SERIES

2. TABLE AND RULE NUMBER FROM AFM 12-50
T10-1, R2, R3

3. CUTOFF INSTRUCTIONS
31 December

4. DISPOSITION INSTRUCTIONS (After Cut Off)
Destroy after 1 year.

FILES DISPOSITION CONTROL LABEL

AF FORM JUL 69 82

Figure 3–6. Sample of AF Form 82.

In this section we will point out some ways of avoiding situations as previously described. This can be done by practicing good laboratory ethics and good interpersonal relations. Ethics is defined as that science which studies the morality (right conduct) of human acts through the method of natural reason. In other words, ethics teaches us how to judge accurately the moral goodness or badness of any human action.

Everyone, except a hermit, has a need for good interpersonal relations. We laboratory techs, whose work brings us into close contact with the public, have the additional need to know and practice good public relations. Perhaps nowhere in the Air Force is the need for good public relations more evident than in our career specialty. Not only do we meet the public, but we meet them when they are at a disadvantage. Since they are usually patients, their cordiality may be a little under par. Thus, we have the responsibility for assuring a cordial and concerned atmosphere, not only throughout the laboratory, but also throughout the hospital.

027. State ways to make the new laboratory specialist or technicians feel wanted in terms of relief of anxieties, acquaintance with the department, and with job instructions.

Orientation of New Personnel. One of the *most lasting* impressions that your fellow technicians and subordinates will have of you is the first one you give them the day they enter the laboratory for that first interview with you as a new worker. Even though new specialists or technicians may be qualified in their speciality, they would appreciate any assistance making them aware of the local conditions in order for them to perform their jobs effectively and with confidence.

On the workers' first assignment, they have even more fears than the experienced workers because more is unknown. Usually, orientation of new workers is done at more than one level of supervision. For instance, as the department supervisor, you may brief workers on the mission and layout of the organization, and an intermediate supervisor may brief them as to the specific job. New workers will be filled with questions about the base, the town, and the new job. You must cater to the workers' personal interests and make them feel a welcome, useful, desirable part of your group.

Make new workers feel wanted. How do you make them feel like a wanted part of your organization? Well, a good way to start is to show a genuine interest in them. Discuss their background and interests with them. You may be able to help if they have housing or transportation problems. Since many military people are often in a financial bind when they report to a new duty station, you may be able to suggest a local source of assistance. The more quickly they can get settled, the sooner they will be productive.

Relieve their anxiety. New workers are interested in the organization they are joining and want to know what job they will be doing. When briefing them, you should describe the function of your department and show them how it is organized. Assign them to positions as quickly as you can. This not only makes them feel needed, but also relieves their anxiety about what job they are going to get. Explain the relation of their work to that of others so that they can see what role they play in the big picture. Let them know to whom they are responsible and who is responsible to them.

Acquaint new workers with the department. Remember that new workers are strangers in the medical unit. Take them around and introduce them to the people they will be dealing with in the organization. Let each person know what job the new workers will be doing, and tell the new workers the function of each person in the unit. This is also a good time to show new workers the layout of the facility. Show them where they can hang their hats and coats and where the water fountain, latrines, etc., are located. If they are eating lunch on base, arrange for someone to go with them the first day. All these acts help make them feel like a part of the organization. Also, explain the local policies to the new workers. These include duty hours, lunch period, breaks, use of the telephone, scheduling leave, duty uniform, etc. Let them know what the policies are so that they will have no doubt whether they are doing right or wrong.

Provide job instructions. You must be sure that new workers get the instructions to do the job. You may provide the instruction yourself or assign the workers to a qualified trainer. Provide the learning aids and equipment needed, check frequently on progress, encourage questions, make corrections, and give encouragement. Getting new workers started on the right track is important to their future.

Exercises (027):

1. How can you make your new workers feel like a wanted part of your organization?

2. What can you do to help relieve the anxiety of new workers?

3. What are some ways through which you can acquaint new workers with the department?

4. How can you be certain that your new workers will perform the job that is expected of them?

028. Cite the significance of maintaining confidentiality in terms of its effects upon good professional relationship, patient relations, and relationship with laboratory coworkers.

Professional Working Relationship. As a medical laboratory technician, you should maintain a good professional working relationship with your supervisor, coworkers, patients, doctors, nurses, and all other hospital personnel. This is essential to the delivery of high quality patient care and service. In other words, you must practice the highest standard of integrity in and out of the laboratory primarily in dealing with the patients and ultimately all other hospital personnel. Your "sense of right and wrong" must be such that your behavior and motives are above suspicion. Let's begin with the confidentiality you should maintain for the patients' concerns and your coworkers.

Patients' confidentiality. As a medical laboratory technician, you have access to information about patients that may be confidential in nature. You may learn that Mrs. Bailey is undergoing treatment in the mental health clinic. You may even note a lab report on one of her teenage sons showing Gram-negative intracellular diplococci from a urethral discharge. After the morning chemistry runs have been completed, you note that her glucose tolerance test shows an abnormal curve. Well, what do you do about it? Do you take these juicy tidbits of information to the club or to the ball or bowling games and blab about them to your buddies? The answer to that is a most definite NO! There is never any justification for reporting information outside of the "need-to-know" group. Confidentiality should be stressed in your laboratory.

An administrative specialist who was filing laboratory reports noticed that his neighbor was an inpatient. He looked and saw that the neighbor was having cosmetic surgery and proceeded to spread the news around the base. The patient was deservedly upset. She complained to the hospital commander and threatened to sue the Air Force. The best that could be done was to sympathize with the patient and assure her that the incident would not, under any circumstance, happen again and that corrective action would be taken. These and many other similar incidences of loose talk, coupled with a poor patient relationship, can most certainly bring a lawsuit.

Which individuals possess the right to know about the patient? If you performed a blood glucose on a patient, and the result was grossly elevated, whether or not the patient is your friend or coworker, the person's physician should be the first to know the result. Thus, any results of laboratory tests that may influence the treatment of the patient should first be referred to that physician. When the information may indicate a detriment to the patient's ability to perform his or her duties, the patient's supervisor should be notified by the attending physician.

Coworkers' confidentiality. It is of great importance that you, as a team member or leader, have the trust and confidence of your coworkers and subordinates. Let them know and feel that you trust them and their work. If your technicians fouled up a special chemistry test, correct them in private. Don't go around telling everyone in the lab how dumb Sergeant Foster and Airman First Class Swift are.

Keep their faults in strictest of confidence; tell them privately. An open and frank expression of what you think and how you feel about each work situation will be an excellent start toward increasing interpersonal trust and confidence. Your technicians will trust you, learning will take place, and confidence between you and your coworkers will be reciprocal.

You must have confidence in your subordinates. Some supervisors lack confidence in subordinates because they feel that they are too young to do a particular job. Others lack confidence or at least refuse to assign subordinates certain types of work because they feel they are of the wrong sex, race, or religion. There is no job that cannot be done by a woman as well as a man, or by a young person as well as an older person.

Exercises (028):

1. When is there ever a justification for reporting patient information outside the "need-to-know" group?

2. What possible serious action can a patient take when his or her privacy has been violated because of loose talk or a situation resulting in a poor patient relationship?

3. After completing the LDH, CK, and CK–MB on the NCOIC, the results were elevated. Immediate notification of the results should be made to whom?

4. When the test results indicate that the patient's condition may affect his or her ability to perform his or her duties, whom should the physician notify?

5. If you want to have the trust and confidence of your laboratory coworkers and subordinates what should you do?

6. When your subordinates make errors in laboratory tests, how should you correct them to increase interpersonal trust and confidence among your workers?

029. Cite the significance of providing quality laboratory service in terms of its effect on patient care, laboratory results, and lab responses in emergencies and competency in the laboratory.

Service. The hospital is a service organization. This means that we provide services to those who need it. Sometimes there is a difference of opinion as to just who needs it. Meanwhile, keep in mind that we provide service to the patient, not the other way around. Another way of stating it is that "the patient is always right until proven wrong."

Some of the services you can perform for your patients are quite evident. These services may be inferred from your job description. Other services—those little "extra goodies"—may not be quite so evident, yet definitely contribute to a smoother operation in the medical laboratory. Let's take a look at some of these "extra goodies."

Delayed care. Sometimes the laboratory is understaffed and you can't get to a patient immediately. Don't ignore your patient. Take the time to inform your patient as to the reason for the delay. The patient will be inclined to be more understanding when hw or she knows what is going on. Use discretion. Don't try to displace your aggression by telling the patient that the technician is "out looking for his spouse" or is "making a BX run."

Lab or clinic errors. Verify your laboratory results before the report leaves the laboratory. Always take the few seconds necessary to doublecheck and correct all discrepancies. Nothing is more embarrassing than for the doctor to hospitalize a patient with a hematocrit of 25 vol% and Hgb of 8.5 g when you read the wrong specimen because the specimen was misnumbered or mislabeled. Stay on top of your STATs. If you can discover these errors before they leave the lab or the patient leaves the hospital, the doctor should be grateful and so should the patient.

STATs, ASAPs. If has often been said that the real emergencies are few and far between, but there are certain times when some lab work is more urgent than others. Thus, if patients, through no fault of their own, need STAT laboratory work, don't jump all over them.

In a situation where you are running behind and trying to get caught up with your lab work you should, as a professional, quickly and accurately process the lab work, then when the workload slows down, go see the physicians(s) requesting the laboratory tests. Tactfully and intelligently explain your situation in the laboratory. As another professional, the doctor should be willing to explain the purpose of his or her acute concern. If necessary, the doctor may delay some tests for the next day when he or she is made aware of the limited capability for handling some types of laboratory work at specific times, if this is the case. Remember, you could be "wearing his or her shoes." What would you have done if the patient's health had been in your hands and you had the responsibility?

Competency. Basically, this means to know your job and how to do it. If you don't know what you are doing, even the best snow job in the world won't fool the patients for long. If there are some areas in which you are unsure of your proficiency, ask your boss for help. He or she will be glad to help you in the interest of better laboratory efficiency. The competency which you and your coworker strive to attain and maintain insures an overall proficiency in the clinical laboratory and a better quality of patient care and service.

Exercises (029):

1. When laboratory service to patients must be delayed (for example, patient waiting for blood to be taken in waiting room, or completion of STAT urinalysis or CBC), and this condition is due to personnel shortage or some unforeseen emergency, what action can you take to insure credit for maintaining good laboratory service?

2. After completing a series of STAT CBCs, one of the patients suspected of having a high white count, with symptoms of right lower quadrant pains, had a white count of 8,900/mm^3, a hematocrit of 27 vol% and a Hgb of 9.0 grams. The patient was admitted for observation, and the results reported within 6 hours indicated a white count of 18,500/mm^3, a hematocrit of 45 vol% and Hgb of 15.0 grams. What was the most probable cause of the discrepancy?

3. How can you prevent such types of errors as given in exercise 2 to insure continued quality service in your laboratory?

4. A series of STAT or CBC, BUNS, glucose electrolytes, and cardiac enzymes have been requested. Due to a shortage of lab personnel for that day you appear to be falling behind and cannot get caught up with your requests as you would desire. Your OIC, or supervisor is not available for assistance. What action should you take that might help alleviate the situation in the future?

5. What does competency mean?

6. A better quality of patient care and service, and an overall proficiency in the laboratory is guaranteed when all laboratory techs strive to maintain and attain ____________.

030. Indicate the effects on public relations of patient-centered behavior by laboratory personnel.

Patient-Centered Behavior. The way you, as a medical laboratory technician, behave makes your reputation; it affects the attitudes of those people who use your medical facility. You earn your own favorable or unfavorable reputation. Your behavior and reputation have either a favorable or unfavorable influence on the attitude of the public. Your medical facility's reputation depends upon your reputation as well as the reputation of every other member of your medical facility's staff.

If you behave in a way to earn yourself a good reputation as a medical laboratory technician, you will have a favorable influence on the public's attitude. Remember that patients, as well as those people with whom you work, have needs that must be satisfied. Medical facilities exist to provide health care services to people. Act to satisfy patients' needs first. Let patients know by your actions that your medical facility exists for them. Remember that patients may feel that their survival needs are threatened and therefore behave in a discourteous and demanding manner. Keep cool. Be courteous and helpful. By satisfying your patients' needs, you and the other members of your medical facility's staff will gain a fine reputation and can thereby effectively satisfy your own needs.

You are often the first and last contact that the patient has with your medical facility. First and last impressions are lasting. So know your job. When patients feel that you know your job, they tend to have an acceptable opinion of your service to them. When you can't deliver a service to a patient, explain why. People accept an action (or a delay) better when they believe that there is a good reason for it.

Exercises (030):

1. What does the reputation of your medical facility depend upon?

2. Whose needs must be satisfied first—your own, the staff's, or the patient's?

031. For given situations, specify the proper telephone technique to be used and the precaution to be observed for communications security in the clinical laboratory.

Telephone Technique. Patients are influenced not only by personal contact with medical laboratory personnel, but also by the presence or lack of good telephone technique.

Here are a few rules that should help your telephone manners.

Answer promptly. Don't ignore the ringing telephone. Many patients will not break the connection until they are answered. If you are too busy to carry on a conversation, answer the telephone by saying, "Medical Laboratory, please hold a few minutes." Don't make the caller hold on too long, though. Listening to a silent, forgotten line makes people angry rapidly. If you are too busy to take care of the caller in a minute or so, return to the phone and tell the patient to hold on and that you will return in a specified amount of time. When you finally take the call, thank the patient for waiting.

Identify yourself and your office. Have the courtesy to let the caller know who answered the phone. Give the name of your office, then your name, and offer assistance. It never hurts much to add, "May I help you?"

Speak distinctly. How many times have you called somebody and heard just a mumble as the answerer identified himself or herself? When you speak on the phone, especially on an AUTOVON line, speak distinctly. It will save time and trouble for both you and the person on the other end of the line.

Be prepared to take a message. If the person called is unavailable to answer the phone, take the message on Standard Form 63, Memorandum of Call. Make sure that the message is delivered to avoid embarrassment. If the call is for one of the laboratory officers or NCOIC who is assisting with phlebotomies in the venipuncture section, don't interrupt the patient's treatment, but deliver the message at the earliest opportunity. It is usually better to have the individual in the lab return the call rather than have the patient return to the laboratory to have his or her venipuncture repeated. See figure 3-7 for an example of a properly filled out SF 63. Here are a few quick tips: The OIC or NCOIC is never "busy" but is always "with a patient." This avoids the implication that the person is too busy to speak to the party who is calling. Don't give unnecessary information such as "the OIC is in another office but will be back in a few minutes." Remember that the telephone is not secure, and if you should happen to have some classified information, you should not discuss it on the telephone.

Avoid giving medical advice. Diagnosis over the telephone usually leads to erroneous conclusions. Refer the patient to the proper clinic for treatment. If you are answering questions on lab-related problems, make sure that your information is correct. Refer the patient to the doctor for advice.

Be attentive. Listen to what the caller has to say. Don't try to do two jobs at one time by listening to the phone and trying to do something else at the same time. Both the calling patient and the other job will be downgraded. Take notes if necessary.

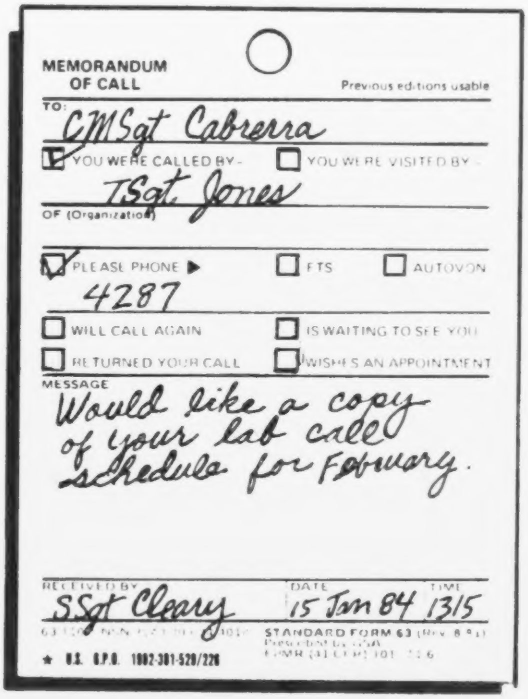
Don't yell into the mouthpiece. If the called party is a little distance from the phone, put the phone down gently (or put it on "hold") and approach the called party telling her or him of the call. Don't put your hand over the mouthpiece and yell down the hall. In addition, if your radio is on, turn the volume down in order to eliminate any background sounds.

Communications security (COMSEC). Remember that your unclassified telephone conversations with your friends and seemingly unrelated unclassified telephone conversations made by other base laboratory personnel can provide an enemy agent bits and pieces of information. In observing communications security (COMSEC), we will protect classified information. For instance, operations, plans, programs, strengths, weaknesses, numbers, equipment, deployments, capabilities, intentions, and just about everything else can be obtained through communications monitoring. Some examples follow.

Communication #1. The NCOIC of an Air Force clinic laboratory makes an AUTOVON call to a former coworker at a medical center laboratory in Europe. He tells them that his workload is increasing and that his techs are drawing 25 specimens a day to be shipped to a specified area laboratory for cholinesterase testing.

Communication #2. A laboratory technician assigned to a base in Turkey calls a coworker assigned to Wilford Hall Medical Center in San Antonio, Texas. She mentions that they are obtaining an increased number of blood cultures. She further states that the organism they are isolating has characteristics similar to *Legionnella pneumophila,* the etiologic agent of Legionnaires disease.

Communication #3. A laboratory specialist assigned to the chemistry section of an overseas medical center laboratory makes a telephone call to a friend at a stateside laboratory. He states the the projected leaves for all base personnel in the laboratory have been cancelled for the next 3 months because of a steadily increasing workload.



MEMORANDUM OF CALL

Previous editions usable

TO: CMSgt Cabrerra

☑ YOU WERE CALLED BY- ☐ YOU WERE VISITED BY-

TSgt. Jones

OF (Organization)

☑ PLEASE PHONE ▶ ☐ FTS ☐ AUTOVON

4287

☐ WILL CALL AGAIN ☐ IS WAITING TO SEE YOU

☐ RETURNED YOUR CALL ☐ WISHES AN APPOINTMENT

MESSAGE

Would like a copy of your lab call schedule for February.

RECEIVED BY SSgt Cleary DATE 15 Jan 84 TIME 1315

STANDARD FORM 63

Prescribed by GSA

★ U.S. G.P.O.

Figure 3–7. Sample of Standard Form 63.

Communication #4. The NCOIC of the laboratory at Loring Air Force Base calls the NCOIC of the laboratory at Hickam Air Force Base. The NCOIC at Hickam tells his friend that he's been requested to extend the hours of the laboratory service and to increase his 2-week lab supply stock levels to 4 weeks and to prepare for an increase in patient load coming from the Far East. He further states that he was thinking of "scrounging" as many vacutainer needles as he can.

Each of the fictitious communications above is unclassified. No one violated security. In each example, the people communicating had no idea that the other conversations had taken place. And yet, we can see from examining all of the conversations together that each call contains bits and pieces of a story—a story that to enemy intelligence analysts would probably be reported to their superiors.

Access to our communications is a simple matter if one has the proper equipment. Telephone calls, for example, can be tapped in so many ways that people using the telephone cannot detect the presence of a tap. The conversations can be recorded and studied by an intelligence analyst.

Exercises (031):

1. Your technician in the drawing room (phlebotomy) is running behind schedule and is with three patients with "hard to get at" veins. The phone rings, and the caller insists upon talking to the technician in the drawing room. What do you do now?

2. A telephone call was referred to the chemistry section from a patient who wanted to find out the results of his glucose tolerance test. As the chemistry department supervisor, what action should you take?

3. Which of the numbered communications in the text suggest each of these avenues of investigation to an enemy intelligence analyst?

   _____ a. Cancellation of projected leaves for all base personnel would suggest some type of defensive or offensive operation.

   _____ b. A type of organism suspected in increased numbers from blood cultures, especially one with an epidemic track record, may lead the enemy to determine how vulnerable the installation will be to outside attacks.

   _____ c. Efforts to increase or the sudden increase of stock levels of supplies may give some indication that preparation is made for some specific purpose which may warrant closer monitoring.

   _____ d. Workers involved in any occupation requiring the use or storage of chemicals that can affect nervous tissue could cause suspicion. The determination of "true" cholinesterase in red cells is of importance only in assessing the exposure to certain potent insecticides related to the "nerve gases."

3-5. Clinical Laboratory Reports and Accreditation Program

What purpose do your clinical laboratory reports serve? On what forms are the reports submitted and where do you get the information to complete them? What is the Uniform Charts of Accounts (UCA)? What are the three types of accounting data that are gathered and three main components in Uniform Charts of Accounts operations? This section discusses the purpose, preparation, and submission of clinical laboratory reports.

032. Cite the primary uses and disposition of AF Form 235, Report of Patients, by the clinical laboratory.

Purpose and Content of Report. The primary uses of Report of Patients data are budgeting and financial planning, manpower and staffing, facilities and equipment procurement, operational capability analysis, and patient management during peacetime and wartime. Data from the Report of Patients are a mainstay for medical resources planning and allocation at all levels of the USAF Medical Service. Data collected and reported by medical treatment facilities are transmitted to the major command and subsequently to Headquarters USAF. You can therefore see why the unquestionable accuracy of your monthly and quarterly report is of prime importance.

AF Form 235, Reports of Patients. The AF Form 235 (fig. 3-8) is prepared and submitted to the medical resources management office on a monthly basis for a reporting period that covers the entire calendar month. You maintain a copy of the report in your laboratory files. You may devise a form for each section of the laboratory on which personnel may enter their daily totals. The form should contain the correct point value. Each section should submit reports at a definite date or cut-off, after which as NCOIC you will verify, complete the figures, and enter them on the AF Form 235, Report of Patients. AFM 168–695, *MAMS - Medical Administrative Management System—Base,* provides adequate guidelines for preparation of AF Forms 235 and 235d. Let's discuss how to transfer the raw data onto the AF Form 235.

REPORT OF PATIENTS	CODE 4871	*REPORT CONTROL SYMBOL*	
REPORTING FACILITY AND LOCATION		PERIOD COVERED 1-31 Jan 84	DATE ENDING 31 Jan 84

I. DAILY AVERAGE STRENGTH SERVED FOR MEDICAL CARE, EAD MILITARY PERSONNEL

LINE		AIR FORCE A	ARMY B	NAVY MARINE C
1	TOTAL			
2	ATCH FOR OUTPNT CARE			
3	ON BASE			
4	OFF BASE			
5				
6				
7	ATCH THROUGH DISP			
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				

II OUTPATIENT AND QUARTERS PATIENT VISITS FOR OBSERVATION, DIAGNOSIS, TREATMENT, FLIGHT OR OTHER COMPLETE PHYSICAL EXAMS

	PERSONNEL CATEGORY	NO. OF VISITS
21	TOTAL	
22	EXTENDED AD MIL	
23	AIR FORCE	
24	ARMY	
25	NAVY-MARINE	
26	OTHER ACTIVE MIL SVC	
27	AD MEMBERS NONMIL UNIF SVC	
28	RETIRED MEMBERS UNIF SVC	
29	DEPN AD MEMBERS UNIF SVC	
30	AIR FORCE	
31	ARMY	
32	NAVY-MARINE	
33	OTHER	
34	DEPN. RET AND DECD MEMBERS UNIF SVC	
35	U.S. CIVILIAN EMPLOYEES	
36	ALL OTHER	

III OTHER VISIT AND PHYSICAL EXAM DATA

		NUMBER
37	FLIGHT PHYSICAL EXAMS	
38	OTHER COMPLETE PHYSICAL EXAMS	
39	IMMUNIZATIONS	
40		
41		

IV DEATHS

LINE	STATUS AT TIME OF DEATH	EXTENDED ACTIVE DUTY			OTHER D
		AF A	ARMY B	NAVY MARINE C	
42	TOTAL				
43	PATIENT, NONBATTLE				
44	PATIENT, BATTLE CASUALTY				
45	CRO, NONBATTLE				
46	CRO, BATTLE CASUALTY				

V NEW, NOT-EPTS, VD CASES TREATED, EAD MILITARY PATIENTS

		GONORRHEA A	SYPHILIS B	OTHER VD C
47	AIR FORCE			
48				
49				
50	ARMY			
51				
52				
53	NAVY-MARINE			
54				
55				

VI DIAGNOSTIC TESTS AND EXAMINATIONS, AND PRESCRIPTIONS

		TOTAL A	IN-PATIENT B	OUTPNT & QTR PNT C
56	X-RAY FILMS EXPOSED			
57	FLUOROSCOPIC EXAMS			
58	ROUTINE 12 LEAD ECG's			
59	OTHER SPEC CARDIOVASC STUDIES			
60	EEG's			
61				
62				
63	OTHER SPEC PULM FUNCT TESTS			
64				
65	REFRACTIONS			
66				
67				
68				
69				
70	LABORATORY PROCEDURES	69692	13724	55968
71	PRESCRIPTIONS			

VII EAD MIL PATIENTS EXCUSED FROM DUTY

	DIAGNOSIS CATEGORY	AIR FORCE A	ARMY B	NAVY MARINE C
72	TOTAL			
73	DISEASE			
74	NONBATTLE INJURY			
75	AIRCRAFT			
76	MOTOR VEHICLE			
77	OTHER			
78	BATTLE CASUALTY			

AF FORM JUL 84 235 PREVIOUS EDITION IS OBSOLETE

Figure 3–8.

Reporting facility and location. Enter the designation and location of your medical treatment facility and office symbol. If applicable, include the name of the air command that has jurisdiction over your medical treatment facility.

Period covered. Enter the number of days in the report month.

Date ending. Enter the last day of the report month.

Section VI, Diagnostic Tests and Examinations, and Prescriptions. Report on line 70 the total number of tests and procedures performed for inpatients and outpatients. Controls, standards, and repeat tests will be included. DO NOT include any laboratory tests or procedures performed in Histopathology, for other hospitals and clinics, or for base medical services not engaged in the treatment or examination of patients.

Exercises (032):

1. What are some primary uses of AF Form 235, Report of Patients?

2. When is the Report of Patients prepared and submitted?

3. Where does the medical treatment facility send the data collected from the Report of Patients?

4. To what office does the laboratory submit the Report of Patients?

5. What Air Force manual provides guidelines for preparation of AF Form 235 and 235d?

6. On AF 235, in Section VI, Diagnostic Tests and Examinations, and Prescriptions, on line 70, the total number of tests and procedures is included for what two general categories?

7. Which laboratory tests and procedures are NOT included on line 70, in Section VI?

033. Cite the purpose, content, and preparation requirements of the AF Form 235d.

AF Form 235d, Report of Patients—Clinical Laboratory Quarterly Report. The AF Form 235d (fig. 3-9) provides clinical laboratory workload and personnel data. The report is prepared at the end of each calendar quarter. Let's take a look at figure 3-9 to see how this report is prepared.

Reporting facility. Enter the designation, location, and office symbol of your medical treatment facility.

Laboratory classification. Enter your laboratory classification (symbol A through D—see classification of laboratories in AFR 160-32, *Clinical Laboratory Classification and Capabilities).*

Period covered. Enter the inclusive period in the 3-month report. For example, note in figure 3-9, 1 Jan—31 Mar 84.

Date ending. Enter the last day of the report month of that quarter.

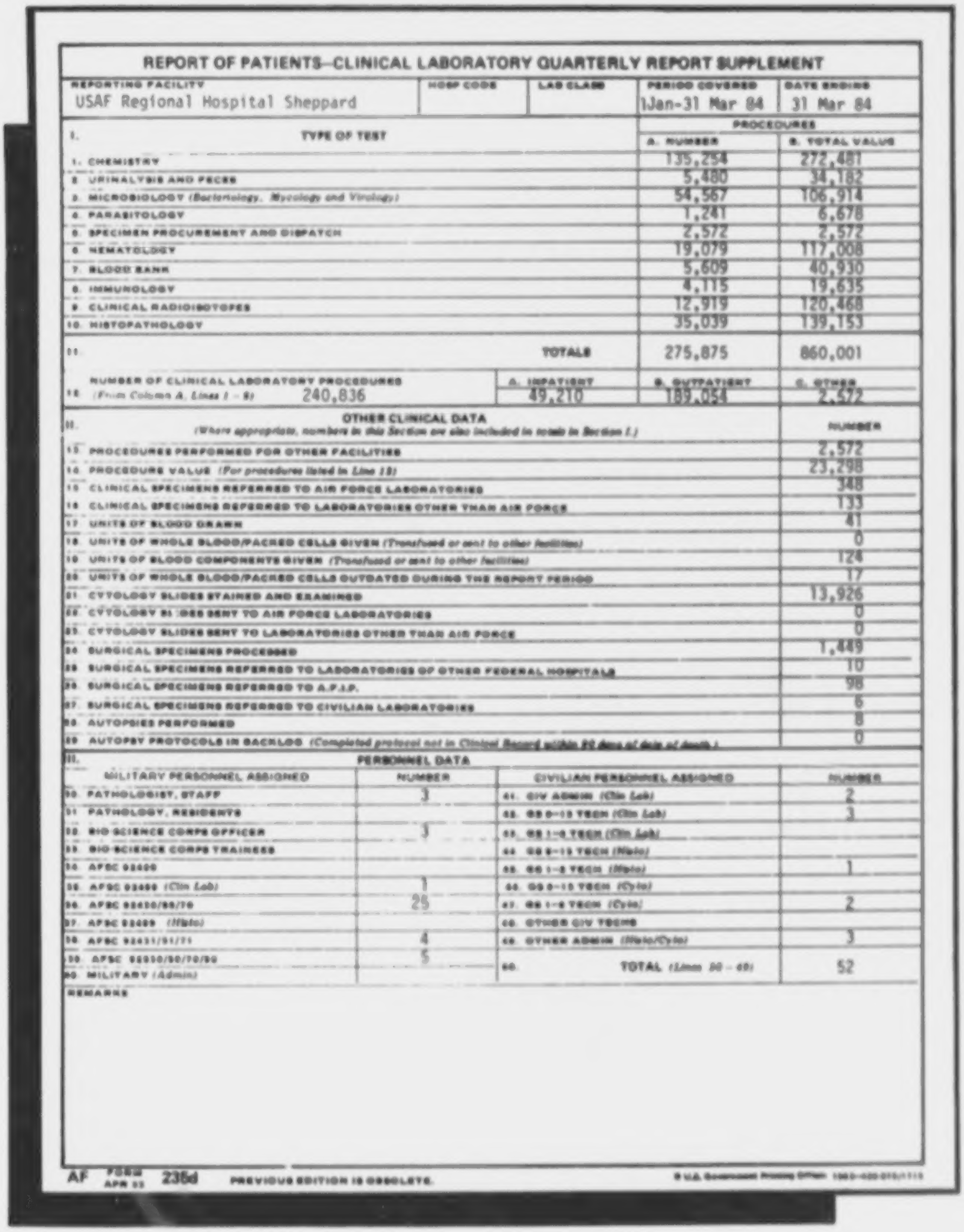
Section I., Type of Test. Enter in column A by category, lines 1-10, the number of procedures, to include standards and quality control procedures, specimen procurement (collection), specimen dispatch, and clerical functions in the procedure totals, regardless of specimen source (for example, inpatient, outpatient, or any other location). Reagent and specimen blanks are not to be counted as separate procedures.

The number of procedures reported are based on the "unit of count" provided in the College of American Pathologists "Laboratory Workload Reporting Method" (LWRM) manual. Each new edition of the LWRM manual and implementation date for its use will be provided by the Air Force Consultant in Pathology (AFIP).

A repeat test or procedure is defined as a test done to solve an unforeseen problem encountered in a patient sample run. To qualify as a repeat test, all of the analytical, data handling, and recording steps following the initial preparation of the specimen must have been performed again. The routine performance of duplicate procedures simply for quality assurance purposes without reasonable probability of discrepant results is not considered to be a problem, and therefore such procedures do NOT qualify as repeats. A repeat constitutes one raw count.

Replicate (duplicate, triplicate, etc.,) is the planned multiple performance of certain steps. Replicates included in a specific methodology are already part of the unit value per procedure and should not be counted as a repeat test. Duplicate analytical testing in the prothrombin time procedure is an example of a test which normally includes replicate testing as an integral step in the routine method and thus does not qualify for any additional raw count credit.

Laboratory personnel should exercise their own judgement as to which line is appropriate for reporting laboratory workload data, except for reporting clinical radioisotope tests. Procedures for which "unit values" are not listed in the master long list of the LWRM manual may be counted in the raw count total only. An arbitrary or locally generated unit value WILL NOT be assigned when none is published in the LWRM manual, and therefore no



REPORT OF PATIENTS—CLINICAL LABORATORY QUARTERLY REPORT SUPPLEMENT

REPORTING FACILITY	HOSP CODE	LAB CLASS	PERIOD COVERED	DATE ENDING
USAF Regional Hospital Sheppard			1Jan-31 Mar 84	31 Mar 84

I. TYPE OF TEST	PROCEDURES A. NUMBER	PROCEDURES B. TOTAL VALUE
1. CHEMISTRY	135,254	272,481
2. URINALYSIS AND FECES	5,480	34,182
3. MICROBIOLOGY *(Bacteriology, Mycology and Virology)*	54,567	106,914
4. PARASITOLOGY	1,241	6,678
5. SPECIMEN PROCUREMENT AND DISPATCH	2,572	2,572
6. HEMATOLOGY	19,079	117,008
7. BLOOD BANK	5,609	40,930
8. IMMUNOLOGY	4,115	19,635
9. CLINICAL RADIOISOTOPES	12,919	120,468
10. HISTOPATHOLOGY	35,039	139,153
11. TOTALS	275,875	860,001

12. NUMBER OF CLINICAL LABORATORY PROCEDURES *(From Column A, Lines 1 - 9)*	A. INPATIENT	B. OUTPATIENT	C. OTHER
240,836	49,210	189,054	2,572

II. OTHER CLINICAL DATA *(Where appropriate, numbers in this Section are also included in totals in Section I.)*	NUMBER
13. PROCEDURES PERFORMED FOR OTHER FACILITIES	2,572
14. PROCEDURE VALUE *(For procedures listed in Line 13)*	23,298
15. CLINICAL SPECIMENS REFERRED TO AIR FORCE LABORATORIES	348
16. CLINICAL SPECIMENS REFERRED TO LABORATORIES OTHER THAN AIR FORCE	133
17. UNITS OF BLOOD DRAWN	41
18. UNITS OF WHOLE BLOOD/PACKED CELLS GIVEN *(Transfused or sent to other facilities)*	0
19. UNITS OF BLOOD COMPONENTS GIVEN *(Transfused or sent to other facilities)*	124
20. UNITS OF WHOLE BLOOD/PACKED CELLS OUTDATED DURING THE REPORT PERIOD	17
21. CYTOLOGY SLIDES STAINED AND EXAMINED	13,926
22. CYTOLOGY SLIDES SENT TO AIR FORCE LABORATORIES	0
23. CYTOLOGY SLIDES SENT TO LABORATORIES OTHER THAN AIR FORCE	0
24. SURGICAL SPECIMENS PROCESSED	1,449
25. SURGICAL SPECIMENS REFERRED TO LABORATORIES OF OTHER FEDERAL HOSPITALS	10
26. SURGICAL SPECIMENS REFERRED TO A.F.I.P.	98
27. SURGICAL SPECIMENS REFERRED TO CIVILIAN LABORATORIES	6
28. AUTOPSIES PERFORMED	8
29. AUTOPSY PROTOCOLS IN BACKLOG *(Completed protocol not in Clinical Record within 90 days of date of death.)*	0

III. PERSONNEL DATA

MILITARY PERSONNEL ASSIGNED	NUMBER	CIVILIAN PERSONNEL ASSIGNED	NUMBER
30. PATHOLOGIST, STAFF	3	41. CIV ADMIN *(Clin Lab)*	2
31. PATHOLOGY, RESIDENTS		42. GS 9-13 TECH *(Clin Lab)*	3
32. BIO SCIENCE CORPS OFFICER	3	43. GS 1-8 TECH *(Clin Lab)*	
33. BIO SCIENCE CORPS TRAINEES		44. GS 9-13 TECH *(Histo)*	
34. AFSC 92400		45. GS 1-8 TECH *(Histo)*	1
35. AFSC 92490 *(Clin Lab)*	1	46. GS 9-13 TECH *(Cyto)*	
36. AFSC 92430/50/70	25	47. GS 1-8 TECH *(Cyto)*	2
37. AFSC 92499 *(Histo)*		48. OTHER CIV TECHS	
38. AFSC 92431/51/71	4	49. OTHER ADMIN *(Histo/Cyto)*	3
39. AFSC 92030/50/70/90	5	50. TOTAL *(Lines 30 - 49)*	52
40. MILITARY *(Admin)*			

REMARKS

AF FORM APR 83 235d PREVIOUS EDITION IS OBSOLETE.

☆ U.S. Government Printing Office: [illegible]

Figure 3-9. Sample of AF Form 235d.

credit will be taken in section I, column B, for such procedures. Any such procedures or tests not listed in the current edition of the LWRM manual should be identified by letter to the Air Force Consultant in Pathology (AFIP). Newly assigned unit values will be distributed by the Air Force Consultant in Pathology as they become available from the College of American Pathologists (CAP).

Special reporting instructions for lines 1–10. (NOTE: Line numbers omitted are self-explanatory).

Line 1. Report clinical laboratory radioisotope procedures on line 9; otherwise, self-explanatory.

Line 3. Report all bacteriology, mycology, and virology on this line.

Line 5. Report all clerical handling, specimen procurement, and specimen dispatch procedures. Specimen dispatch refers only to sending a specimen from the hospital/clinic laboratory to another laboratory that is independent of the shipping laboratory.

Line 9. Report ALL tests and procedures utilizing radioisotopes and performed in the clinical laboratory on this line.

Line 10. Report all surgical pathology, autopsy, and cytology work on this line.

Lines 1 through 10, column B. Enter the total procedure value for all tests and procedures performed and reported in column A, lines 1–10. The procedure value is obtained by multiplying the number of procedures by the unit value for each procedure as listed in the LWRM manual. Procedure value total will be rounded to the nearest whole number.

Line 12, column A — Enter the number of laboratory tests and procedures listed on lines 1 through 9 which were performed for inpatient services. Include controls, standards, and repeats as appropriate.

Line 12, column B — Enter the number of tests and procedures listed on lines 1 through 9 which were performed for outpatient services. Include controls, standards, and repeats as appropriate.

Line 12, column C — Enter the total number of procedures from lines 1 through 9 which were performed for other than inpatients or outpatients. Those procedures performed may include standards, controls, repeats, clerical functions, specimen procurement, and specimens dispatched relating to this work on any specimen coming from from a requesting source not definable as either inpatient or outpatient. Specimens and procedures in this category may come from Veterinary Service, Preventive Medicine, other medical facilities, or other sources. NOTE: Line 12C must equal the total of lines 1–9, column A, minus the sum of column 12A and 12B.

Section II, Other Clinical Data.

Line 13. Report only clinical laboratory procedures performed for other medical facilities. Controls, standards, and/or repeat tests, when run ONLY for the purpose of supporting work from other medical facilities will also be reported on this line. (NOTE: The line 13 total procedure count is also included in the totals of Section I, lines 1–9, column A, above. Therefore, the line 13 total must be equal to or less than the total reported on line 12C of the 235d report.)

Line 14. Report the total procedure value for those tests reported on line 13.

Lines 15 and 16. Report the number of specimens referred to another Air Force laboratory or to other than Air Force laboratories.

Line 17. Report the total number of units of blood collected by the laboratory for hospital or other use.

Line 18. Report the total number of units of whole blood/packed cells either transfused or shipped to another facility.

Line 19. Report the total number of units of components (platelets, cryoprecipitate, ffp, etc.) either transfused or shipped to another facility. Report packed cells on line 18 of 235d report.

Line 20. Report the total number of units of while blood and/or packed cells which are outdated.

Line 21. Report the total number of cytology slides examined, regardless of type or source.

Line 22. Report the total number of cytology slides referred to Air Force Histopathology Laboratories or Air Force Cytology Centers.

Line 23. Report the cumulative total number of cytology slides referred to all other governmental (non-Air Force) and civilian laboratories.

Line 24. Report the total number of surgical pathology specimens processed. A surgical specimen, for the definition of this report, shall be equal to one completed surgical pathology report having a single surgical accession number.

Line 25. Report the number of surgical specimens referred to other Federal laboratories. Refer to "line 24" above for specimen definition.

Line 26. Report the total number of accessioned specimens referred by the histopathology service to the Armed Forces Institute of Pathology (AFIP). Refer to "line 24" above for specimen identification.

Line 27. Report the number of surgical specimens referred to civilian laboratories. Refer to "line 24" for specimen definition.

Line 28. Report the total number of autopsies performed, whether or not the autopsy specimens are processed in the same laboratory.

Line 29. Report the total number of autopsy cases for which a completed protocol is not in the clinical record within 90 days of death.

Section III, Personnel Data. Enter on lines 30 through 50 the number of military and civilian personnel assigned to the laboratory by AFSC classification, as of the last day of the reporting period.

Line 34. Report AFSC 92400 staffing.

Line 35. Report AFSC 92499 (clinical lab only).

Line 36. Report AFSC's 92430/92450/92470. Do not include Phase II clinical lab students enrolled in course J5A2092450.

Line 37. Report AFSC 92499 (histology lab only).

Line 38. Report AFSC's 92431/93451/92471.

Line 39. Report AFSC's 92530/92550/92570/92590.

Line 40. Report military administrative personnel assigned to the clinical laboratory on this line.

Line 41. Report civilian administrative personnel assigned to the clinical laboratory on this line.

Line 42. Report all GS–9 through GS–13 13 civilian technicians working in th clinical laboratory on this line.

Line 43. Report all GS–8 and lower civilian technicians working in the clinical laboratory on this line.

Line 44. Report all GS–9 through GS–13 civilian technicians working in the histology laboratory on this line.

Line 45. Report all GS–8 or lower civilian technicians working in the histology laboratory.

Line 46. Report all GS–9 through GS–13 civilian technicians working in the cytology laboratory.

Line 47. Report all GS–8 or lower civilian technicians working in the cytology laboratory.

Line 48. Report any other assigned civilian technical personnel on this line if they are not more appropriately reported on lines 41 through 46 above.

Line 49. Report all military and/or civilian administrative personnel assigned to the histology and/or cytology laboratory on this line.

Exercises (033):

1. What information does the AF Form 235d provide?

2. What information is included on AF 235d, Report of Patients — Clinical Laboratory Quarterly Report Supplement, column A, by category, in lines 1–10?

3. How are reagents and specimen blanks counted in each test?

4. On the AF Form 235d, the number of procedures reported are based on the "unit of count" provided in what manual?

5. Who will provide each new edition of the manual containing the "unit count" values?

6. What is a repeat test or procedures?

7. A batch of CK run (tests) were repeated because two of the three controls were out of range. Will you count these tests as repeats? Why?

8. Who will distribute the newly assigned unit values when they become available from the College of American Pathologists?

9. What procedures are included in the total of AF Form 235d, line 12, column C, Other?

10. On what line of the AF Form 235d, section II—Other Clinical Data, should the total number of units of whole blood/packed cells, either transfused or shipped to another facility, be reported?

11. How would you define a surgical pathology specimen for the AF Form 235d report?

12. What general information is reported in section III, Personnel Data?

034. Cite the purpose of the Uniform Chart of Accounts (UCA), three main components in UCA operations, and the function of the UCA automated system in terms of its effect on the clinical laboratory workload reporting.

Uniform Chart of Accounts (UCA). In August 1973, at the direction of the President, a military health care study was begun. One area of special interest was evaluating how the three services reported financial and operating performance data. Many differences and inconsistencies were found. As a result, a Tri-Service work group of Air Force, Army, and Navy representatives was formed in July 1976 and tasked to develop a uniform accounting system for resources and performances that supports the military health care system.

The plan developed by the Tri-Service work group is called the Uniform Chart of Accounts (UCA). It establishes uniform reporting procedures for military medical treatment facilities by providing common data element definitions for three types of accounting data that are gathered. These are (1) expenses (supplies, base operations, and the like), (2) personnel utilization (civilian and military), and (3) workload statistics (performance and assignment factors).

Components in UCA operations. There are three components in UCA operations. They are (1) collecting data, (2) compiling and formatting data, and (3) producing management information reports. In most facilities the current procedures for collecting and reporting data are completed by time-consuming manual methods of filling out worksheets and transaction logs.

UCA automated system. Federal Data Systems Corporation (FDSC) has been contracted to develop and install an automated system for collecting and reporting UCA workload data in pathology, the medical laboratory, pharmacy, and radiology departments of military medical treatment facilities. Under this contract, FDSC is applying computer technology to the task of recording the large volume of UCA data. This computerized system is called the Automated Source Data Collection (ASDC) system. It is made up of five subsystems. Three of the subsystems are designed to collect the UCA workload data. They are (1) pathology, (2) pharmacy, and (3) radiology subsystems. The other two subsystems are designed to accept, process, and report the accounting data. They are (1) Automated Source Data Maintenance (ASDM), and (2) Expense Assignment System Preprocessor (EASPP) subsystems.

A primary object of the ASDC system is to reduce the staff hours that medical personnel must spend in recording UCA information and preparing the required reports.

The ASDC pathology subsystem. The ASDC pathology subsystem is easy to operate and tailored to the day-to-day activities performed in the pathology department. Using the same source documents (usually chits) and familiar terminology, pathology (laboratory) personnel will enter the required UCA data at work stations located in their department.

Once the data have been entered, the computer will accumulate the information and produce daily, monthly, and quarterly reports. In most cases these features will eliminate manual tallying of statistics each quarter. Also, through this feature, the College of American pathologists (CAP) workload report data will be available on a timely basis.

Exercises (034):

1. What three types of accounting data are provided through the Uniform Chart of Accounts (UCA)?

2. What are the three main components in the UCA operations?

3. Presently, UCA collecting and reporting is done in which departments of the military medical treatment facilities?

4. What are the two subsystems that are designed to accept, process, and report the accounting data?

5. What is the primary object of the Automated Source Data Collection system?

6. Where would laboratory personnel enter the required UCA data?

7. What are three advantages of having available data entered in the computer?

035. State the purpose, features, and requirements of the College of American Pathologists' Inspection and Accreditation Program as it relates to laboratory inspection and accreditation.

Laboratory Inspection and Accreditation. The College of American Pathologists' Inspection and Accreditation (I&A) Program was developed with the primary objective of improving the quality of clinical laboratory services and assuring the accuracy and reliability of test results. The program is a professional, voluntary, peer-review program. Why is CAP important for laboratory improvement? Let's begin with quality assurance.

Quality assurance. Accuracy and reliability cannot be assured without a comprehensive system of controls to decrease or eliminate the many variables that can effect accuracy. Thus, the quality control refers to a surveillance process in which the performance of personnel, equipment, and materials are observed in some systematic way that provides a record of performance. A significant feature of the I&A Program is that it assures a thorough examination in all areas through the use of the *Inspection Checklist*. The checklist contains more than 2000 questions developed through input from the leading experts in the various subspecialties of laboratory medicine. The checklists are computerized to allow for constant updating as the "state-of-the-art" continues to develop. The CAP's I&A Program is arranged to protect the patient whose doctor orders medical tests and gives assurance that these findings are reliable and that tests were carried out by proper methods and qualified personnel.

Proficiency testing and monitoring. One of the major requirements for achieving and maintaining accreditation is the successful participation in an interlaboratory comparison testing program. Laboratories in the College's I&A Program are required to participate in the proficiency testing programs offered through their survey programs. Accredited laboratories receive a semiannual summary alerting them to any possible problems in specimen identification.

Educational benefits. The CAP's I&A Program inherently provides continuing education, through peer review and professional consultation. The pathologist-inspector is often accompanied by a team of professionals representing the entire range of laboratory personnel, such as doctors, supervisory medical technologists, and administrative personnel. These professionals, along with the pathologist-leader of the team, bring with them the knowledge and experience gained through past inspections, as well as their own professional experiences. This provides both the setting for an exchange of knowledge that is beneficial for both the laboratory being reviewed and the reviewers. The inspector may find, for example, that the laboratory he or she is reviewing has a more efficient method for recording temperatures than the method that the inspector may be using in his or her own laboratory.

The CAP Program is not a pass or fail proposition. It is constructive rather than restrictive. It is an overall educational process to upgrade the quality of laboratory service and maximize the accuracy of results. If a laboratory meets all aspects of the standards, it is accredited. If a laboratory fails to meet all aspects of the standards for accreditation, it is advised on ways to correct deficiencies and encouraged to reapply for inspection when an acceptable standard of quality is achieved. In addition, the College has recently been accepted by JCAH (Joint Committee On Accreditation of Hospitals) so that a hospital-based laboratory that is accredited by CAP is not inspected by a JCAH laboratory surveyor. This results in a cost savings achievement by that facility.

Exercises (035):

1. What is the primary purpose for the development of the College of American Pathologist Inspection and Accreditation Program?

2. What does the surveillance process of quality control involve?

3. What is a given significant feature of the I&A Program?

4. The CAP's I&A Program is arranged for whose protection and in what manner?

5. What is one of the major requirements for achieving and maintaining accreditation?

6. In what way does the CAP's I&A Program provide continuing education through review and professional consultation?

7. If a laboratory fails to meet all aspects of the standards for accreditation, what action is taken?

036. Cite the Air Force Medical Laboratories that should be accredited in terms of conformance with proficiency survey programs.

USAF Laboratory Accreditation. All stateside Class A, B, C, and D, and overseas Class A, B, and C medical laboratories must attain and maintain accreditation with the College of American Pathologists. Each USAF hospital and clinical laboratory should receive a staff assistance visit by a knowledgeable biomedical laboratory officer or clinical pathologist biannually.

USAF Laboratory Proficiency Test Programs. Funding for monitoring the level of proficiency in all Air Force Medical Service laboratories will be available from central procurement as authorized by the Surgeon General's Office, Headquarters USAF. Each laboratory is expected to offer a diversity of laboratory tests to comply with the minimum standards established in AFR 160-32, *Clinical Laboratory Classification and Capabilities*. Each and every proficiency specimen unknown that corresponds to a test offered in individual laboratories will be tested.

Inspection checklist. As a medical laboratory technician, you should be aware of the existence of the *Inspection Checklist* as it affects the laboratory in general and the specific section of the laboratory to which you are assigned. Minimum standards have been prepared by the Commission on Laboratory Accreditation of the College of American Pathologists for the purpose of improving the quality of pathology service. Bear in mind that the guiding principle in the preparation of these standards has been to be constructive rather than restrictive. Thus, the checklist serves to amplify and explain the standards and to indicate specific items that will be investigated. Its primary concern is: how well does this facility serve the patient under the prevailing local conditions? The *Inspection Checklist* is completed for every inspection. All questions must be answered as "yes," "no," or "not applicable." Each question is assigned a "phase" category. A *Phase 0* question is "for information only." *Phase I* deficiencies represent items that are considered important in the management of an outstanding laboratory service;

therefore, they should be corrected if possible. Items listed as *Phase II* represent deficiencies and must be corrected before accreditation can be granted. Such deficiencies can directly affect patient care and seriously affect the mission of that facility. Checklists to include the extent of services provided by the laboratory are available for Section I, *Laboratory General;* Section II, *Hematology;* Section III, *Clinical Chemistry;* Section III-A, *Urinalysis;* Section IV, *Microbiology;* Section V, *Blood Bank;* Section VI, *Diagnostic Immunology and Syphilis Serology;* Section VII, *Nuclear Medicine* (In Vitro and Radioimmunoassay Procedures); Section VIII, *Anatomic Pathology and Cytology;* Section IX, *Cytogenetics;* Section X, *Clinical Histocompatibility*.

Other Proficiency Survey Programs. If additional survey programs are necessary for use by your laboratory, local funds can be used to participate in these programs. All USAF medical laboratories are directed to subscribe to the State Health Department's syphilis serology survey of their respective state, if they are not participating in the CAP Syphilis Serology Survey Program.

Exercises (036):

1. Which Air Force medical laboratories are required to attain and maintain CAP accreditation?

2. Funding for monitoring the proficiency levels in all Air Force Medical Service laboratories will be available from what source?

3. Each laboratory is expected to offer a diversity of laboratory tests to comply with the minimum standards established in what Air Force regulation?

4. What is the guiding principle in the preparation of the standards of the Inspection Checklist?

5. What is the primary concern regarding the function of standards of the Inspection Checklist?

6. What do items listed as Phase II represent?

7. What do items listed as Phase I represent?

3-6. Reference Centers

You may be assigned to a large clinical laboratory where many extensive procedures and special tests are performed or to a small laboratory where many of the chemistry procedures are semiautomated. However, it is indeed rare to find a laboratory that is completely self-sufficient, where no special diagnostic procedures are referred. With the dynamic advancements being made in laboratory medicine, the quantity of tests now available to medical diagnosis and therapy are numerous.

037. Identify the reference centers in terms of the type of services, consultation services, training and technical assistance, and special clinical laboratory procedures that they provide to Air Force and other armed services clinical laboratories.

Consultant Service. To provide diagnostic and consultation services to Armed Forces hospitals, the Surgeon General and the Armed Forces Institute of Pathology (AFIP) Director have designated histopathology centers to serve their hospitals. Thus, these centers provide histopathological and consultant services for Air Force medical treatment facilities in their geographic area. Table 3-3 lists the designated Air Force histopathology centers both within the continental United States and overseas. Any USAF hospital or laboratory with a board-certified pathologist as chief of pathology may become a direct contributor to the AFIP, subject to the coordination with the AFIP Director and the USAF Surgeon General. The histopathology centers serve to:

a. Provide histopathological facilities and diagnostic services for medical facilities that are not adequately equipped or staffed to do this work.

b. Provide regional consultation and training services in pathology and related subjects for medical facilities within designated areas.

c. Review pathological material and records from medical facilities in their region and send reports promptly to the concerned facility.

d. Study all pathology records before forwarding to the AFIP. Refer only cases that will be of further value for reference followup, education, of research.

e. Forward all completed autopsies promptly to the AFIP and materials from such surgical cases as may have future administrative, scientific, or followup value. Surgical cases with tumor-type conditions and those requiring final or confirmatory diagnosis are of particular importance.

Table 3-3 also lists the overseas designated histopathology centers which will provide histopathologic services for those medical facilities within geographic areas which are neither equipped nor staffed to do this work.

***The Armed Forces Institute of Pathology*.** This institute serves as the central laboratory or pathology for the Department of Defense and certain other Federal agencies.

TABLE 3-3

DESIGNATED HISTOPATHOLOGY CENTERS - CONTINENTAL UNITED STATES AND OVERSEAS

Continental United States

David Grant USAF Medical Center, Travis AFB, CA 94535
USAF Medical Center Keesler, Keesler AFB, MS 39534
Malcolm Grow USAF Medical Center, Andrews AFB, MD 20331
USAF Medical Center Scott, Scott AFB, IL 62225
Wilford Hall USAF Medical Center, Lackland AFB, TX 78236
USAF Medical Center Wright-Patterson, Wright-Patterson AFB, OH 45433
USAF Regional Hospital Carswell, Carswell AFB, TX 76127
USAF Regional Hospital Eglin, Eglin AFB, FL 32542
Ehrling Bergquist USAF Regional Hospital, Offutt AFB, NE 68113
USAF Regional Hospital Fairchild, Fairchild AFB, WA 99011
USAF Regional Hospital MacDill, MacDill AFB, FL 33608
USAF Regional Hospital March, March AFB, CA 92508
USAF Regional Hospital Maxwell, Maxwell AFB, AL 36112
USAF Regional Hospital Minot, Minot AFB, ND 58710
USAF Regional Hospital Shaw, Shaw AFB, SC 29152
USAF Regional Hospital Sheppard, Sheppard AFB, TX 76311

Overseas

USAF Hospital Lakenheath, APO New York 09179
USAF Hospital Torrejon, APO New York 09283
USAF Hospital Wiesbaden, APO New York 09220
USAF Hospital Clark, APO San Francisco 96274
USAF Hospital Yokota, APO San Francisco 96328

It is located on the grounds of the Walter Reed Army Medical Center in Washington DC. The functions of this institute are to:

a. Maintain a consultation service for the diagnosis of pathologic tissue for the Department of Defense, for other agencies, and for civilian pathologists.

b. Serve as the reviewing authority of pathologic tissue for the Army, Navy, and Air Force.

c. Conduct research in the broad field of pathology.

d. Provide instruction in advanced pathology and related subjects.

e. Train qualified and selected enlisted personnel of the Armed Forces in pathologic techniques, medical photography, medical arts, and museum activities.

f. Donate or loan educational material to Federal and civilian medical services.

g. Operate the American Registry of Pathology as a cooperative enterprise in medical research and education.

h. Maintain a medical illustration service.

USAF Epidemiological Services. The USAF epidemiological services are established to prevent and control diseases among Air Force military and civilian personnel and eligible dependents. The USAF Medical Service is able to maintain a viable readiness posture through the continuous practice of preventive medicine. The major overall missions of the three units providing epidemiological services are to provide professional consultation and referral capability, conduct epidemiological surveys and investigations, and perform specified analytical procedures in support of USAF medical activities. AFR 161-12, *USAF Epidemiological Services*, gives specific information concerning the organization and responsibilities of each of the following three organizations providing epidemiological services. They are:

a. The Epidemiological Division, USAF School of Aerospace Medicine (AFSC). Mailing address: USAFSAM/EP, Brooks AFB TX 78235.

b. The Fourth Medical Services Squadron (USAFE). Mailing address: 4 MSES, APO NY 09332.

c. Environmental Health Services, First Medical Service Wing (PACAF). Mailing address: 1 MSEW, APO San Francisco 96274.

Other laboratory support facilities. AFR 161-40, *Joint Utilization of Certain Armed Forces Medical Laboratory Facilities*, describes the uses and locations of Army, Navy, and Air Force laboratories, health and environment units, disease vector ecology, and control centers. It also describes the various types of services available and outlines procedures for their utilization. The Army and Navy medical laboratories assist the Air Force in performing all types of clinical laboratory procedures. You should become familiar with all the facilities available within your geographic region. Utilizing such available facilities can enhance the quality of laboratory services you provide with reference to efficiency, economy, and timesaving effort for laboratory results. Table 3-4 lists these joint laboratory facilities and the general services they provide.

TABLE 3-4
JOINT ARMED FORCES MEDICAL LABORATORY FACILITIES

FACILITY	TYPES OF SERVICE
A. US Army Clinical Laboratories 1. Brooke Army Medical Center, Fort Sam Houston, Texas 78234 2. Dwight David Eisenhower Army Medical Center, Fort Gordon, GA 30905 3. Fitzsimons Army Medical Center, Denver, CO 80240 4. Letterman Army Medical Center, San Francisco, CA 94129 5. Madigan Army Medical Center, Tacoma, WA 6. Tripler Army Medical Center, APO San Francisco 96438 7. Walter Reed Army Medical Center, Washington, DC 20012 8. William Beaumont Army Medical Center, El Paso, TX 79920	All types of clinical laboratory and forensic toxicology procedures. Examination of meat, dairy products, and other foods.
B. US Army Medical Laboratories 1. 10th Medical Laboratory, APO New York 09180 2. US Army Medical Laboratory, APO San Francisco 96343	All types of clinical laboratory and forensic toxicology procedures and tests. Examination of meat, dairy products and other foods. The medical laboratory in Europe may be called upon to conduct entomological occupational health, radiological hygiene and environmental engineering and sanitation surveys.

TABLE 3-4 (Cont'd)

FACILITY	TYPES OF SERVICE
C. US Army Environmental Hygiene Agency Commander, USAF Army Health Services Command ATTN: HSPA-H, Fort Sam Houston, TX 78234	Provides worldwide support to the health and environment programs through consultations, supportative services, investigations, and training in entomology, pesticide sciences, occupational health, radiological hygiene, environmental sciences and engineering toxicology, environmental chemistry, and related laboratory sciences.
D. US Army Environmental Health Engineering Agency, Pacific Commander, US Army Environmental Health Engineering Agency, Pacific, Box 243, APO San Franscisco 96331	Provides support to health and environment programs through consultations, supportive services and investigations. Conducts entomological, industrial hygiene, radiological hygiene, environmental and sanitation surveys in WESTPAC and Hawaii.
E. The Epidemiology Consultant Service (EPICON) Walter Reed Institute of Research (WRAIR), Walter Reed Medical Center, Washington, DC 20012	The central epidemiological resource for the US Army EPICON will furnish assistance in investigation and control of significant outbreaks of disease and services to installations throughout the world which normally cannot be provided by other military resources. EPICON & scope includes infections, diseases, chronic diseases, nonbattle injuries, public health aspects of disaster relief operations, and the design of medical studies and research protocols involving application of epidemiological methods.

TABLE 3-4 (Cont'd)

FACILITY	TYPES OF SERVICE
F. US Navy Clinical Laboratory The National Naval Medical Center, Bethesda, MD 20014	Performs all types of clinical laboratory procedures and tests, as well as examination of meat, dairy products and other foods.
G. US Navy Environmental Health Center 3333 Vine St. Cincinnati, OH 45220	Performs environmental and biological chemical analysis related to occupational health exposures.
H. US Navy Environmental and Preventive Medical Units Officer in Charge Navy Environmental Preventive Medicine Unit: (1) Number 2, Norfolk, VA 23511 (2) Number 5, Naval Station, Box 143, San Diego, CA 92136 (3) Number 6, Box 112, FPO San Francisco, CA 99610 (4) Number 7, FPO New York 09521	Provide epidemiological investigations, special environmental health and sanitation surveys, and performs supporting laboratory examinations.
I. US Navy Disease Vector Ecology and Control Centers Officer in Charge Navy Disease Vector Ecology and Control Center Naval Air Station Alameda, CA 94501 or Jacksonville, FL 32212	Provides special surveys and training, consultation, advice and recommendations in matters of vector ecology, control, and prevention.

TABLE 3-4 (Cont'd)

FACILITY	TYPES OF SERVICE
J. US Air Force Elements	Based upon the capability of the facility, USAF units will provide epidemiological investigations surveys such as entomological, occupational health, environmental health, radiological health and other special surveys.
1. Epidemiological Division, USAFSAM (AFSC) Brooks Air Force Base, TX 78235. The principal USAF epidemiological Unit, expertise is available in infectious and chronic diseases, non-battle injury, public health aspects of disaster relief and similar operations, and the design and conduct of studies involving the application of epidemiological methods.	
2. Environmental Health Division, USAF Hospital, Wiesbaden, APO New York 09220	
3. Medical Technical Services, Headquarters First Medical Service Wing (PACAF) APO San Francisco 96274	
4. USAF Environmental Health Laboratory, Kelly Air Force Base, TX 78241	
5. USAF Environmental Health Laboratory, McClellan Air Force Base, CA 95652	

Centers for Disease Control (CDC). The Centers for Disease Control serve as the national focus for developing and applying disease prevention and control, environmental health, and health promotion and health education activities designed to improve the health of the people of the United States. These centers are located in Atlanta, Georgia. They provide many services that are available to the Armed Services, to state, local, international, and private organizations, and to other nations. Services available to Air Force laboratories include the following:

a. In the Clinical Chemistry Division.

(1) Develops and evaluates tests for detecting diseases.

(2) Evaluates techniques, materials, reagents, and devices used.

(3) Furnishes laboratory services and consultation to, and conducts joint programs with, other CDC components.

(4) Provides laboratory training and evaluation.

b. Center for Infectious Diseases.

(1) Conducts surveillance, investigations, and studies of bacterial diseases to define disease etiology and development, effective methods for diagnosis, prevention, and control.

(2) Provides reference/diagnostic services.

(3) Performs research and development on sexually transmitted diseases, including gonorrhea, syphilis, chancroid, granuloma inguinale, *Gardnerella,* and chlamydial and mycoplasmal infections.

(4) Conducts studies and research in diagnosis or actinomycotic and fungal diseases.

(5) Conducts surveillance, investigations, and studies in parasites, vector-borne viral diseases and plague, and other viral and rickettsial diseases.

In addition, the Division of Laboratory Training and Consultation administers a national laboratory training program directed toward transferring current laboratory technologies to state public health laboratories and the nation's laboratory community. Through proficiency testing results and other assessment methods, they identify laboratory problems that can be corrected by training. Additional detailed information is available from the mailing address:

U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control
Atlanta GA 30333

Exercises (037):

1. Match the following reference center in column B with the services they provide in column A. Each element in column B may be used once, more than once, or not at all.

Column A

______(1) Provides histopathological facilities and diagnostic services for medical facilities that are not adequately equipped or staffed to do this work.

______(2) The principle USAF epidemiological unit; expertise is available in infectious and chronic diseases, nonbattle injury, public health aspects of disaster relief, and similar operations.

______(3) Serves as the national focus for developing and applying disease prevention and control, environmental health, and health promotion, and health education activities.

______(4) Are established to prevent and control diseases among Air Force military and civilian personnel and eligible dependents.

______(5) Provides primarily consultant and histopathological services for Air Force medical treatment facilities in their geographic area.

______(6) Provides regional consultation and training services in pathology and related subjects for medical facilities within designated areas.

______(7) Serves as the central laboratory of pathology for the Department of Defense and certain other Federal agencies.

______(8) Trains qualified and selected enlisted personnel of the Armed Forces in pathologic techniques, medical pathology, medical arts, and museum activities.

______(9) Provides professional consultation and referral capability, conducts epidemiological surveys and investigations, and performs specified analytic procedures in support of USAF medical activities.

______(10) Describes the uses and locations of Army, Navy and Air Force laboratories, health and environment units, disease vector ecology and control centers.

______(11) Conducts surveillance, investigations, and studies of bacterial diseases to define disease etiology and development, effective methods for diagnosis, prevention, and control.

______(12) Provides for the development and evaluation of tests for detecting diseases, evaluation of techniques, materials, reagents, and devices used.

______(13) Performs research and development on sexually transmitted diseases, including gonorrhea, syphilis, chancroid, granuloma inguinale, *Gardnerella* and chlamydial and mycoplasmal infections.

______(14) Administers a national laboratory training program directed toward transferring current laboratory techniques to state public health laboratories and the nation's laboratory community.

______(15) Through proficiency testing results and other assessment methods, identify laboratory problems that can be corrected by training.

Column B

a. Armed Forces Institute of Pathology.
b. Histopathology centers.
c. USAF Epidemiological Services.
d. Centers for Disease Control (Clinical Chemistry Division).
e. Centers for Disease Control (Center for Infectious Disease).
f. Epidemiological Division, USAFSAM (AFSC), Brooks AFB TX 78235.
g. USAF Environmental Health Laboratory, Kelly AFB TX 78241.
h. USAF Environmental Health Laboratory, McClellan AFB CA 95652.
i. AFR 161-40, *Joint Utilization of Certain Armed Forces Laboratory Facilities*.
j. Centers for Disease Control (Division of Laboratory Training and Consultation).

Review of Basic Chemistry

CHEMISTRY is the science dealing with the composition of matter and the changes in composition which matter undergoes. Because it is such a tremendously large subject, chemistry is divided into many specialized fields—inorganic chemistry, dealing with the elements and mineral matter; organic chemistry, dealing with carbon compounds originating from living matter or from synthetic sources; physical chemistry; biochemistry; clinical chemistry; and other fields.

The prime function of the clinical chemistry laboratory is the measuring of chemical changes in the body in the interest of diagnosis, therapy, and prognosis of disease. As the technician, you must analyze the various chemical constituents in blood, urine, and other fluids or tissues. It is possible to become quite proficient in a procedure or the operation of automated equipment used in the analysis of some constituents such as serum, blood, or cerebrospinal fluid; but it is equally important to understand the chemical changes in the process. If you have relatively little knowledge of the procedure, in terms of the chemical reaction or principle involved, you are limiting your potential as an Air Force chemistry technician.

You should have some knowledge of chemical structure and use this information to work with chemical formulas and equations. You must be able to define the basic parts of an atom—protons, neutrons, and electrons—and you must be able to determine the relative reactivity of an element by its configuration of electrons. You should know what determines the mass of an element and how this is related to isotopes. You should be able to determine precisely the differences in ions, molecules, compounds, and mixtures and to explain the concepts of valence, oxidation number, chemical bonding, and polarity. With this knowledge, you can write chemical formulas and reactions.

This chapter presents a simple review of basic chemistry and is designed to encourage further study and research where needed. You will find that the material outlined in this chapter represents a deductive approach, which begins with simple basic concepts and proceeds to more complex specific procedures outlined in later chapters.

4-1. Atomic Theory

If you have studied chemistry in the past, you may have found it difficult, or it may have been a snap for you. Even though you found it difficult, you already know a great deal about chemistry that the greatest scientists did not know and understand many years ago. So let's take a brief look at a few basic definitions.

038. Identify the components of the atoms and the molecules in terms of their classification and characteristics.

Look at figure 4-1. Briefly study the makeup of the helium atom, both the semirealistic and diagrammatic examples shown.

Atomic Structure. Basic units of matter are called *atoms*, from the Greek "atomos," which means undivided. The key to the behavior of atoms is their structure. It has long been established that matter is composed of discrete unit particles, a fact outlined with remarkable clarity in the early 1800s by John Dalton, an English physicist. Nuclei of all atoms (except the lightest isotope of hydrogen) contain *neutrons* and *protons*. Today we know, through X-ray and other studies, that atoms are not indivisible but consist of positively charged *nuclei* with electrons revolving around them. We now define the atom as the smallest particle of an element capable of taking part in a chemical change. Atoms are the building blocks of all elements and therefore of all compounds and mixtures as well.

The Molecule. A molecule is the smallest particle of an element or compound having all its chemical and physical properties. Molecules of an element contain one or more atoms of the *same* element, while molecules of a compound contain two or more atoms of different elements. When a molecule of an element consists of only one atom, then an atom and a molecule of that element are identical.

Molecules of elements. Elements may be classified as monoatomic, diatomic, or triatomic, according to whether there are one, two, or three atoms to each molecule.

a. Monoatomic. A molecule of helium (He) contains one atom of helium. The helium atom and molecule are therefore identical. Most elements fall into this category.

b. Diatomic. Each molecule of oxygen (O_2) contains *two* atoms of oxygen. The oxygen atom (O) and the oxygen molecule are not the same. They have very different chemical and physical properties. O_2 is a stable, colorless, odorless gas, while the atom is unstable and immediately combines with other atoms. Hydrogen, nitrogen, fluorine, and chlorine are also diatomic elements.

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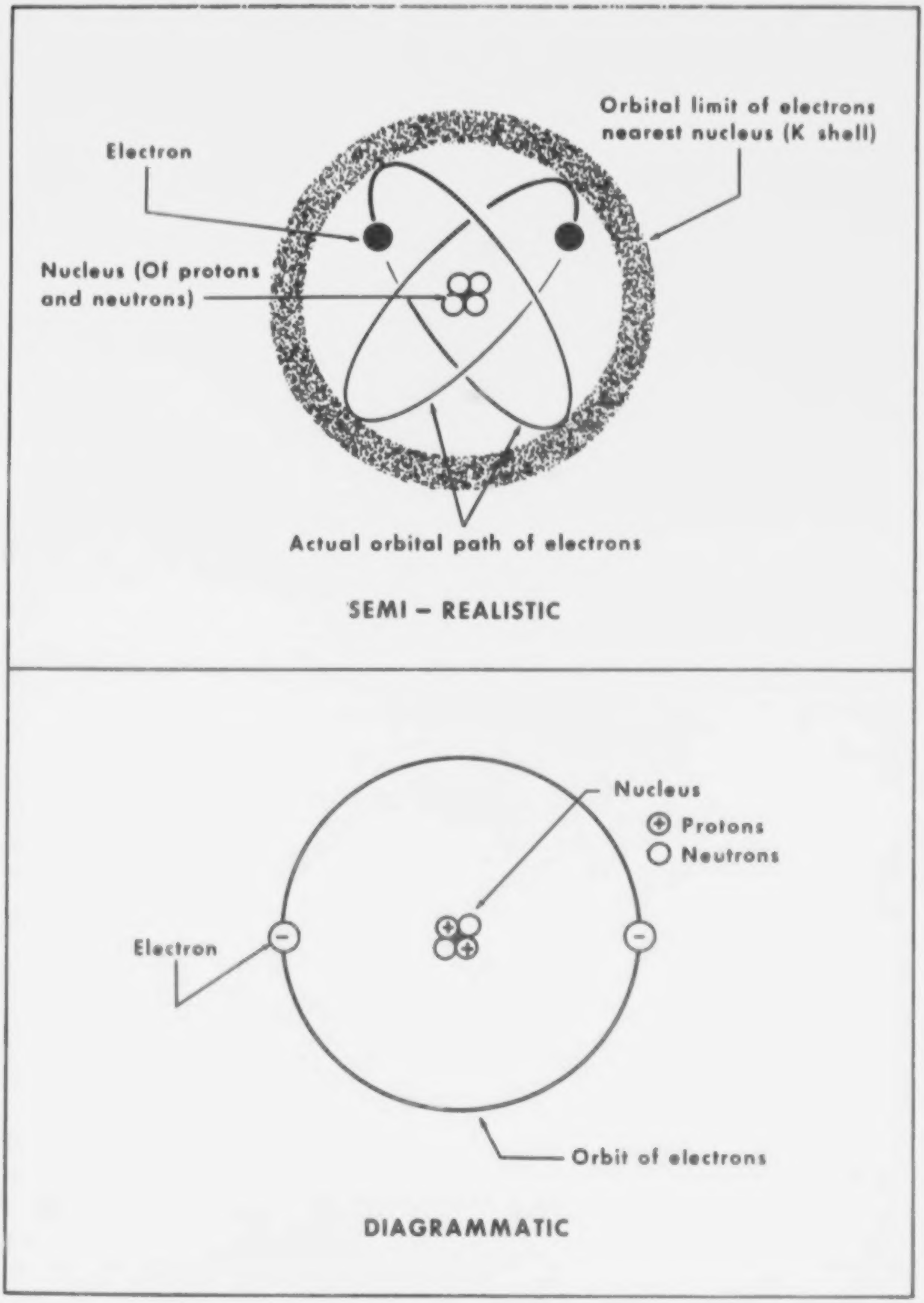

Orbital limit of electrons nearest nucleus (K shell)
Electron
Nucleus (Of protons and neutrons)
Actual orbital path of electrons
SEMI – REALISTIC
Nucleus
⊕ Protons
○ Neutrons
Electron
Orbit of electrons
DIAGRAMMATIC

Figure 4–1. Makeup of the helium atom.

c. Triatomic. A molecule of ozone (O_3) contains *three* atoms of oxygen. Molecules of ozone have different properties (it is an unstable, bluish gas with a pungent odor) than either oxygen molecules or atoms.

Molecules of compounds. When two or more atoms of different elements chemically combine, they form a single unit. This unit is a molecule of the new compound and is the smallest particle of the compounds that can exist. A molecule of water (H_2O) contains three atoms: one of oxygen and two of hydrogen (H). A molecule of sodium chloride (NaC1) contains only two atoms: one of sodium (Na) and one of chlorine (C1). A molecule of sulfuric acid (H_2SO_4) contains seven atoms: two of hydrogen, one of sulfur (S) and four of oxygen.

Subatomic Particles. The atom is made up of three kinds of particles: protons, neutrons, and electrons. Since these are fundamental particles making up all atoms, they are assigned *arbitrary* unit charges and masses.

Proton. A proton(p) is a particle with a positive charge of 1 and a mass of one atomic mass unit (amu). One atomic mass unit is equal to one-twelfth of the mass of an atom of carbon isotope 12_C. The absolute value of 1 amu = 1.66041×10^{-27} kg. However, for our convenience we will use the whole number 1 for all of our calculations. Protons are located in the nucleus of the atom. All atoms of an element have the same number of protons in their nuclei.

Neutron. A neutron (n) has the same mass as that of a proton (one amu), but has no electrical charge. Like the proton, it is located in the nucleus of the atom. The neutrons of an atom are believed to account for the degree of stability that its nucleus has.

Electron. An electron (e^- or .) is a particle with a negative charge of one. It has a mass of only 1/1846 that of a proton. For this reason we are usually able to ignore its weight when finding the total weight of an atom. Electrons very rapidly circle the nucleus of an atom in what are called electron shells or energy levels. They are responsible for determining how an atom will react during a chemical change.

Exercises (038):

1. With the exception of the lightest isotope of hydrogen, the nuclei of all atoms contain what two particles?

2. Define the atom.

3. Define a molecule.

4. Why is nitrogen considered to be a diatomic element?

5. How would you classify a molecule of hydrogen (monoatomic, diatomic, or triatomic)?

6. How many atoms are contained in the following molecules of compounds?

   a. P_4O_{10}.

   b. H_2SO_4.

   c. NaC1.

7. What type of electrical charge does a proton possess and how many atomic mass units (amu)?

8. What type of electrical charge does a neutron have and what is believed to be its purpose?

9. What type of charge does the electron have?

10. Through what media do electrons rapidly circle the nucleus of an atom?

11. What purpose does the electron serve?

039. Explain the arrangement of the electrons within the energy shells in terms of their significance to the atom's chemical properties and the characteristics of the atom with respect to the electrical neutrality, atomic number, atomic mass, isotopes, and atomic weight.

Electron Configuration. The electrons of an atom are arranged in a definite pattern within the various energy levels or shells. Those shells are assigned letters, starting with the lowest energy level (the first one from the nucleus), the K shell, and moving outward, the L, M, N, etc. Each shell is also given a number n; for the K shell n = 1, for the L shell n = 2, for the M shell n = 3, and so forth.

TABLE 4-1
ENERGY SHELL BY ALPHABETICAL DESIGNATION

Electron Shell	Letter	K	L	M	N	...
	n number	1	2	3	4	...
maximum # of e		2	8	18	32	...

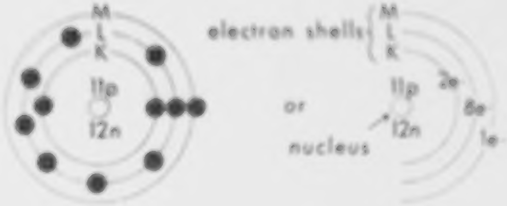


Figure 4-3. Electron configuration of sodium.

To find the maximum number of electrons that each shell can hold we use the formula:

Maximum number of e^- in the n shell $= 2n^2$.

Using this formula, we get the information shown in table 4-1. Note figure 4-2. Generally the electrons fill or partially fill the lower energy levels before starting to fill higher levels. For example, in drawing the atomic configuration of sodium, atomic number 11, there are 11 protons and 12 neutrons in the nucleus. Eleven electrons are found circling about this nucleus. The first two enter the K shell and the next 8 go into the L shell. The one remaining electron must go into the M shell, giving sodium the structure shown in figure 4-3.

The outermost shell containing electrons is called the valence shell, and the electrons within it are termed "valence electrons." The valence electrons are responsible for an atom's chemical properties and will be discussed further under chemical bonding.

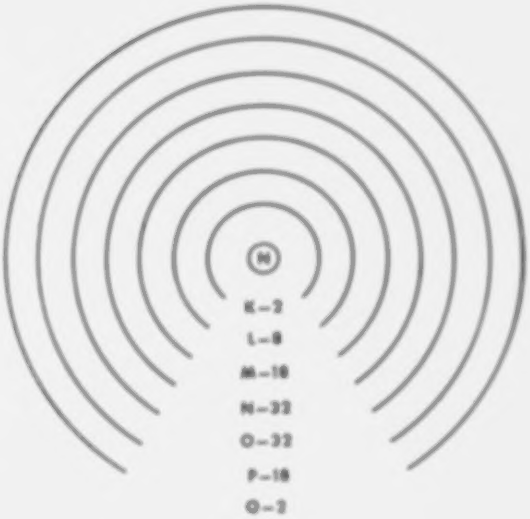


Figure 4-2. Energy shells by alphabetical designation.

Characteristics of the Atom. The present picture of the atom is that of a very heavy, compact, positively charged center, called the nucleus, with the negatively charged electrons rapidly circling it in their shells. The simplest atom is hydrogen. Its nucleus consists of a single proton and is surrounded by one electron in the K shell. The helium atom has two protons and two neutrons in the nucleus surrounded by two electrons.

Electrical neutrality. All uncombined atoms are electrically neutral. As you may recall, this means that there must always be an equal number of electrons (−) and protons (+) in an atom. When atoms gain or lose electrons, they become charged particles called *ions*. Ions are formed during chemical changes and have different properties from the atoms they are derived from. They will be mentioned in more detail in later sections of this chapter.

Atomic number. The atomic number or Z number of an atom is the number of protons found in the nucleus. In the above examples, the atomic numbers for hydrogen and helium are 1 and 2, respectively, as shown in figure 4-4. There are always the same number of electrons in orbit around the nucleus as there are protons in the nucleus if the atom is electrically neutral. Thus, in neutral atoms, these atomic numbers also represent the orbital electrons.

Atomic mass. The mass of an atom can be found by adding up the masses of all the particles in it. We have seen already, however, that an electron's mass is small enough to ignore. Therefore, the atomic mass (expressed in amu) is merely the sum of the number of protons plus neutrons:

Atomic Mass $= p + n$.

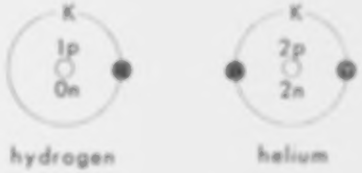


Figure 4-4. Structure of hydrogen and helium.

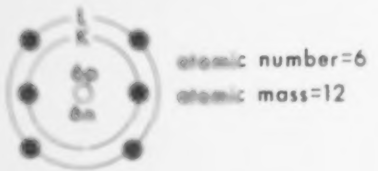


Figure 4-5. Carbon.

Thus, for hydrogen the atomic mass is one, and for helium it is four $(2_p + 2_n)$. A diagram of the carbon atom is shown in figure 4-5. Carbon contains 6 protons (atomic number = 6), 6 neutrons, and 6 electrons and has an atomic mass of 12.

Isotopes. Isotopes are atoms of the same element having the same atomic number but *different atomic masses*. Since the atomic number is the same, these atoms have the same number of protons in their nuclei. They must therefore *differ in the number of neutrons* present. The three known varieties of hydrogen atoms are shown in figure 4-6. Note that the atomic number in each case is 1 (therefore they are all hydrogen atoms), but the atomic mass varies from 1 to 3. All three are considered isotopes of hydrogen, not just the last two, which are the rarer types. Also, since the number of electrons is always the same, isotopes will have the same chemical properties. Chlorine has two isotopes, as shown in figure 4-7. Both isotopes are normally mixed together whenever chlorine is found in nature.

Atomic weight. The atomic weight of an element is defined as its weight relative to that of the carbon—12 isotopes, which is taken to be 12,000. Thus, helium, which weighs about one-third as much as carbon, has an atomic weight of 4.0026. There are several differences between atomic mass and atomic weight:

a. Atomic mass refers to one atom of an element, while the atomic weight refers to the element itself (many atoms).

b. The unit of atomic mass is the amu, while atomic weight is expressed in grams.

c. The atomic mass must be a whole number. The atomic weight is the *average* weight of all isotopes and will therefore not be an exact whole number.

You may refer again to figure 4-7 to further illustrate this point. We shall use chlorine as an example of these differences. As we have seen, there are two chlorine isotopes having *atomic masses* of 35 and 37 amu. The *atomic weight* of the element chlorine is 35.453 grams. This *fractional atomic weight* comes about because chlorine exists as a mixture of about 75 percent of 35-isotope chlorine and 25 percent of 37-isotope chlorine. Thus, 35.453 represents the average weights of the chlorine isotopes, based upon the percentage present.

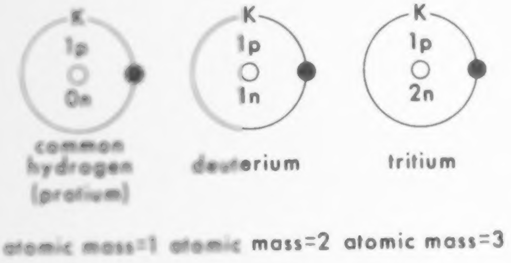


Figure 4-6. Isotopes of hydrogen.

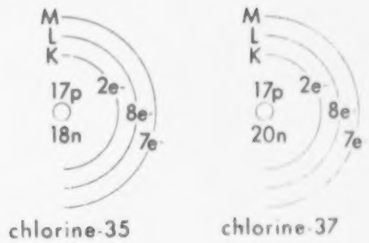


Figure 4-7. Isotopes of chlorine.

The advantage of using atomic weight instead of atomic mass is that we can easily weigh out 12 grams of carbon, but to weigh out 12 amu of carbon could present a major problem.

Chemical Symbols. A chemical symbol consists of a letter or a pair of letters, representing one atom of an element. A chemical symbol can also stand for the element itself or one atomic weight of the element. A symbol is written as a capital letter followed by a small letter if it is necessary. Symbols are derived from the English or Latin name of the element.

You should become familiar with the list of common elements and their symbols, given in Appendix F, Sections 1 and 2.

Exercises (039):

1. What is the maximum number of electrons that can occupy the M shell of an atom?

2. What is the maximum number of electrons that can occupy the N shell if the N number is 4?

3. If there are 6 electrons in the carbon atom, how many electrons will there be in the L shell?

4. What is the outermost electron shell called?

5. What are the electrons within the outermost shell called and for what are they responsible?

6. When atoms gain or lose electrons, they become positively charged particles called ________.

7. During what type of changes are ions formed and how do they compare with the atoms from which they are derived?

8. What is the atomic number?

9. If the atom is electrically neutral, what is the relationship of the number of electrons in the orbits around the nucleus to the number of protons in the nucleus?

10. What is the atomic mass unit (amu)?

11. What are isotopes?

12. What is atomic weight?

13. The unit of atomic mass is the ________, while atomic weight is expressed in ________.

14. What does a chemical symbol consist of and what does it stand for?

4-2. Combination of Elements

Most of the reagents you use in the laboratory will be made from combinations of elements called compounds. In order to use these compounds, you must know certain chemical and physical properties that govern the elements in a compound and the compound itself in its reactions. Concepts such as atomic weight, oxidation number, chemical bonding, and valence are essential for this understanding. In addition, you must be thoroughly familiar with the language of chemistry, symbols, formulas, and equations. It will be necessary for you to memorize the most common symbols and valences listed in this section. Since you will also require this knowledge in other chapters of this volume, study this material so that you will retain these basic concepts and definitions.

040. Distinguish between compounds and mixtures and cite the chemical laws and theories that explain bonding and oxidation numbers.

Compounds. A chemical compound is a substance made up of two or more elements, chemically united in definite proportions by weight. Its properties are different from those of the elements from which the compound was formed. Table salt is a compound, made up of the elements sodium and chlorine. Water is also a compound, made up of the elements hydrogen and oxygen.

Mixtures. A mixture is composed of two or more elements or compounds that have been physically mixed. Unlike a compound, the typical mixture has no chemical reaction taking place between its parts. Each element or compound does not lose its original chemical properties. Generally, mixtures can be separated into individual compounds by physical means. Water added to a salt-and-sand mixture will dissolve the salt. By filtering the mixture we can remove the sand, and heat will evaporate the water, leaving the salt.

Molecular Composition. Compounds have a definite molecular composition by weight. Water is an example of a compound, and it can be decomposed by electrolysis. Upon such decomposition using an electric current, it is found that 88.81 percent of the water by weight is oxygen and the remainder is hydrogen. It is a law in chemistry that the quantity of one element needed to combine with another element is a fixed ratio by weight for any given compound. This is referred to as the *law of definite proportions*. In other words, a fixed weight of one element will combine with a fixed weight of another element. Sometimes an element can combine to form different compounds in simple multiples of this fixed weight. For example, the weight of oxygen in hydrogen peroxide (H_2O_2) is twice the weight of oxygen in the compound water, but the weight of hydrogen is the same. When the law of definite proportions is expanded to explain the occurrence of elements in compounds as a simple multiple of their occurrence in other compounds, it is termed the "law of multiple proportions."

Bonding Theory. Compounds are formed from elements by bonding when an atom fills its valance shell. You will recall that the valence shell is the outermost electron shell. The valence shell is said to be "complete" or "satisfied" when it has either zero or eight electrons in it (except for the K shell which is complete with two electrons).

Rule of eight. Our entire bonding theory is based upon the rule of eight, which is as follows: *Atoms always* *combine in such a way as to complete their valence shells.* They can do this by gaining, losing, or sharing electrons. The only exception to this rule is the K shell, which is satisfied with only two electrons, since this is the maximum number it can hold. Atoms that already have eight electrons in their valence shell are inert. They do not form compounds.

Valence. "Valance" is defined as the number of electrons gained, lost, or shared by an atom when it satisfies its valence shell–or simply as the combining power of an element. The valence may be either positive or negative, depending upon whether the atom has lost or gained electrons. The sum of the valences of all the atoms in a compound must be zero, since compounds like atoms are electrically neutral. For example, the hydrogen atom can give up or share an electron in order to complete its valence shell. When it gives up its one electron, we say that it has a valence of +1. The oxygen atom must borrow or receive two electrons in order to complete its valance shell. When it receives two electrons, we say that it has a valence of −2. If hydrogen and oxygen combine, we have a molecule of water, which is electrically neutral. Valence may be determined from a knowledge of valence shell population, from knowledge of specific compounds which the elements in question form, or from a periodic chart (Appendix F, Sections 1 and 2). For a list of the common elements, radicals, and their common valence states, refer to table 4-2.

Ions. When an atom loses or gains electrons, it becomes an ion. An ion, therefore, is a charged atom. A positively charged ion is called a *cation*, and a negatively charged ion is called an *anion*. The attraction between oppositely charged ions is what binds the compound together.

Oxidation number. In reactions where there is a loss or gain of electrons, the term "oxidation number" is more meaningful than "valence." The oxidation number has a positive or negative value, depending on whether electrons were lost or gained, respectively, in the reaction. For example, in the reaction between hydrogen and chlorine in the formation of hydrochloric acid, the oxidation number of the element hydrogen is +1 because of its loss of an electron to chlorine in the formation of HC1. The element chlorine has in this reaction acquired an oxidation number of −1. Thus, we see that the oxidation number is a dynamic concept which refers to the expression of the valence potential of elements in specific compounds. For certain elements in particular compounds, the oxidation number of an element may vary. For example, iron with a *Potential* valence of +3 has an oxidation number of +2 when it is in compounds such as ferrous sulfate. Since oxidation numbers depend upon how electrons are associated with the individual atoms in the various compounds, some rules may be necessary to correctly identify the oxidation number.

The rules governing oxidation numbers are as follows:

(1) The sum of the oxidation numbers of all atoms in a molecule is always 0.

(2) Oxygen usually has an oxidation number of −2 (one exception is H_2O_2, in which seven electrons are associated with the oxygen portion of the molecule).

(3) In practically all compounds, metals have a positive oxidation number.

(4) The element hydrogen almost always has an oxidation number of +1 because it commonly forms compounds with more electronegative elements. Hydrides are an exception.

(5) The halogens usually have an oxidation number of -1 except when they are in combination with oxygen.

(6) Elements in the free state, such as H_2 or N_2, always have an oxidation number of 0.

Exercises (040):

1. How do compounds differ from mixtures?

2. When mixed, alcohol and water intermingle and produce heat. The water and alcohol can be separated by distillation. Is this a mixture or a compound that is formed?

3. What is the law of definite proportions?

4. How are compounds formed according to the bonding theory?

5. Using the bonding theory based upon the rule of eight, atoms always combine in such a way as to complete their valence shells through wat processes?

6. What is meant by "valence"?

7. What binds the compound together?

8. Since compounds, like atoms, are electrically neutral, what must be the sum of the valence of all the atoms in a compound?

9. What is the oxidation number of free nitrogen?

TABLE 4-2
THE MOST COMMON VALENCES OF SOME ELEMENTS AND RADICALS

A. Positive Valences (Electron Donors)

+1	+2	+3	+4
Ammonium -- NH_4	Calcium -- Ca	Aluminum -- Al	Carbon -- C
Cuprous -- Cu	Cupric -- Cu	Bismuth -- Bi	Manganic -- Mn
Hydrogen -- H	Ferrous -- Fe	Ferric -- Fe	
Mercurous -- Hg	Lead -- Pb	Nitrogen -- N	
Potassium -- K	Magnesium -- Mg		
Silver -- Ag	Manganous -- Mn		
Sodium -- Na	Zinc -- Zn		

B. Negative Valences (Electron Acceptors)

-1	-2	-3
Acetate -- $C_2H_3O_2$	Carbonate -- CO_3	Arsenate -- AsO_4
Bicarbonate -- HCO_3	Dichromate -- Cr_2O_7	Nitrogen -- N
Halogens -- Cl, Br, I, F	Oxygen -- O	Phosphorous -- P
Hydroxide -- OH	Permanganate -- MnO_4	Phosphate -- PO_4
Nitrate -- NO_3	Sulfite -- SO_3	
	Sulfate -- SO_4	

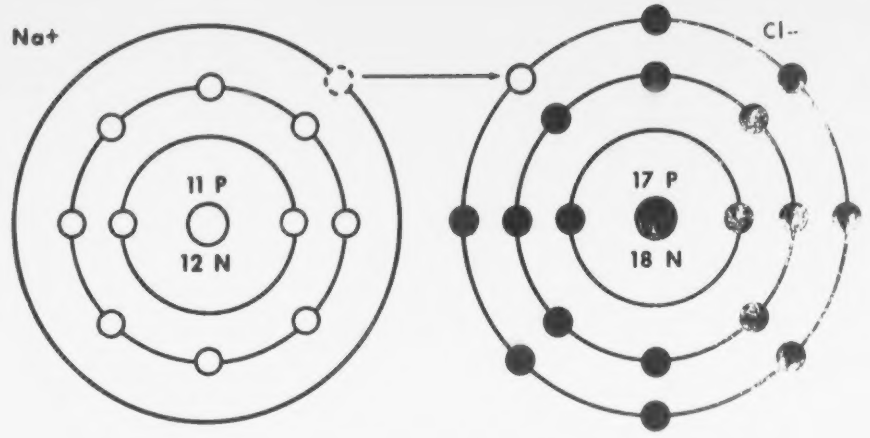


Figure 4-8. Electrovalent bonding in the NaCl molecule.

10. What is the exception to the rule of eight?

041. Identify the types of chemical bonds and terms related to chemical bonding.

Types of Bonding. It is acceptable to categorically describe types of bonding; however, it should be pointed out that no compound is formed exclusively by one type of bond.

Electrovalent (ionic) bonding. Electrovalent or ionic bonding occurs when atoms form compounds by transferring one or more electrons. The atoms combining in this manner must be very different in their physical and chemical properties (for example, a metal reacting with a nonmetal). The metal loses one or more electrons, and the nonmetal gains these electrons. The end result is that all atoms having their valence shells satisfied. In electrovalent bonding, electrons from the valence orbit are transferred from one atom to another. A compound is formed from the two elements bound together by the electrovalent bond. This occurs in the reaction between sodium and chlorine. Sodium reacts with chlorine to yield sodium chloride (NaCl). As shown in figure 4-8, sodium has only one electron in its outermost orbit; chlorine has seven. In seeking stability, chlorine needs eight electrons in the outer orbit. Note in figure 4-8 that the single valence electron of the sodium atom is given up and accepted into the valence shell of chlorine. The compound formed now has eight electrons in the outer orbit and is stable. Elements that differ significantly in electronegativity values tend to form electrovalent (ionic) bonds.

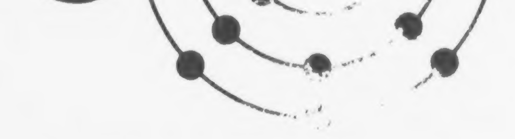


Figure 4-9. Covalent bonding in the HCl molecule.

Covalent bonding. In covalent bonding, each atom donates one or more valence electrons to be shared equally by the two. An example of covalent bonding is the reaction between hydrogen and chlorine. Hydrogen chloride in the pure (gaseous) state is covalent, but hydrochloric acid is an electrovalent compound in water. An atom of hydrogen reacts with an atom of chlorine to yield a molecule of HCl. Figure 4-9 shows that the hydrogen donates one valence electron and the chlorine seven to make a covalently bonded stable compound with eight electrons in the outermost orbit.

Coordinate covalent bonding. The coordinate covalent bond is essentially the same as the covalent bond except that one atom donates all the electrons to be shared. An example of coordinate covalent bonding is represented by the compound formed from sulfur and oxygen (fig. 4-10). In this reaction, the sulfur atom provides two electrons to each of three atoms of oxygen. But only one oxygen atom contributes to the octet of the sulfur valence shell. A compound with eight valence electrons in the outer ring—sulfur trioxide—is formed by coordinate covalent bonding.

Multiple valence. Some elements, cobalt for instance, can lose or gain more than one amount of electrons due to suborbitals. Let's look at the above example, cobalt, more closely. Its atomic number is 27. Therefore, its electron configuration is 2, 8, 15, 2. Since there are two electrons in the outer shell that could be lost, we would expect this atom to have a valence of +2. However, it can also have a +3 valence, because sometimes it loses an electron from the subshell in the next to the outer shell. This occurs because the energy of the subshell is close to the energy of the valence shell. An atom is fairly stable in one of these forms or the other but can be caused to gain or lose the subshell electron. When it gains the electron it is said to be REDUCED. That is, the valence is reduced from +3 to +2. The opposite can also occur. When an atom loses an electron or electrons, it is said to be OXIDIZED. These processes are illustrated below, using cobalt as an example.

REDUCTION: $\mathrm{Co}^{+3} + e- \longrightarrow \mathrm{Co}^{+2}$ (reduced cobalt)

OXIDATION: $\mathrm{Co}^{+2} \longrightarrow \mathrm{Co}^{+3} + e^-$ (oxidized cobalt)

Since it is not always easy to predict the valence, or *oxidation state,* a list of valences is provided in the Periodic Chart of the Elements (Appendix F, Section 2).

Exercises (041):

1. Match the terms related to chemical bonding in Column B with their appropriate definitions in column A. Some column B items may be used once, more than once, or not at all.

Column A

_____ (1) The type of bonding that occurs when atoms form compounds by transferring one or more electrons.
_____ (2) The type of bonding that occurs when each atom donates one or more valence electrons to be shared equally by the two.
_____ (3) The type of bonding in which one atom donates all the electrons to be shared.

Column B

a. Multiple valence.
b. Covalent bonding.
c. Electrovalent bonding.
d. Coordinate covalent bonding.
e. Ionic bonding.
f. Reduction.
g. Oxidation.

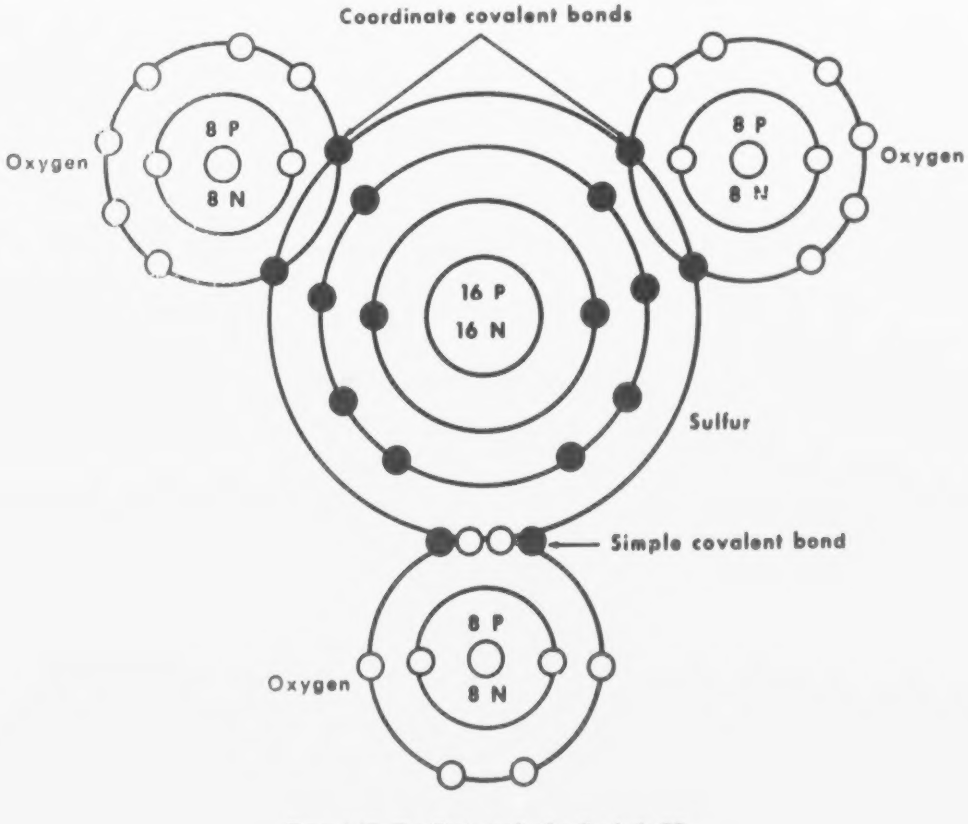


Figure 4-10. Coordinate covalent bonding in the SO_3.

Column A

_____(4) The ability of some elements to lose or gain more than one amount of electrons due to suborbitals.
_____(5) Gaining of an electron.
_____(6) Losing of an electron.

4-3. Chemical Formulas, Reactions, and Equations

The significant feature of using atomic symbols, such as S for sulfur and Fe for iron, is that they provide the chemist with a shorthand way to write formulas and reactions. In this section, you will review the procedures for writing chemical symbols, formulas, and equations and refresh your knowledge of chemical reactions.

042. Write the chemical formulas for given compounds and cite characteristics of radicals.

Chemical Formulas. By now you should be familiar with the common symbols that are used to represent the elements. This information can always be obtained from a table of elements, as shown in Appendix F, Section 1. The problem, then, becomes how to use such symbols in writing formulas. Formulas are the combinations of symbols representing compounds. Compounds are named from the formula, and the formula is derived on the basis of the valences of the two or more elements entering into the reaction. The valances of two or more elements combining chemically to form a compound determine the formula for the compound formed. The compound must be electrically neutral and, therefore, must have as many negative charges as positive. A chemical formula consists of a symbol or group of symbols, with proper subscripts, representing one molecule or the weight of one molecule of an element or compound.

Radicals. Radicals are groups of covalently bonded atoms that react as a single unit and pass from one compound to another unchanged. In the following example water reacts as hydrogen ions, H^+, and hydroxyl radicals, OH^-. Notice that it does not react as two H^+ ions and one oxygen ion, O^{--}.

$$2\,Na + 2\,H_2O \longrightarrow 2\,NaOH + H_2$$

Radicals behave as do single ions and are assigned overall valences as are ions. A list of common radicals and their valences will be found in Appendix F, Section 2.

Most radicals contain oxygen, which is indicated by using an *-ate* or *ite* suffix after the name. The -ate ending indicates a radical with the most oxygen, while the -ite ending is a radical with one less oxygen. For example:

NO_3^{-1}	Nitrate	SO_4^{-2} Sulfate
NO_2^{-1}	Nitrate	SO_3^{-2} Sulfite

Also, many of the radicals form new radicals by covalently bonding hydrogen to them. When this occurs, the new radical is named by using the prefix *bi-*. Some examples include:

CO_3^{-2}	Carbonate	SO_3^{-2} Sulfite
HCO_3^{-1}	Bicarbonates	HSO_3^{-1} Bisulfite

Classification of compounds. Compounds can be classified according to the number of elements present; however, simple compounds are named by a binomial (two-name) system. The first name is for the positive part, and the second for the negative part of the compound.

Binary compounds. Binary compounds possess only two elements in their molecules and have an *-ide* ending. Examples include sodium chloride (NaCl), barium bromide ($BaBr_2$), and calcium oxide (CaO).

Tertiary compounds. Tertiary compounds have three elements present in their molecules, usually with a radical included. Examples are sodium sulfate (Na_2SO_4), potassium sulfite (K_2SO_3), and calcium carbonate ($CaCO_3$).

Quaternary compounds. Quaternary compounds contain four elements to each molecule. Again a radical is usually present. Examples include sodium bicarbonate ($NaHCO_3$), ammonium sulfate [$(NH_4)_2SO_3$], and trichloroacetic acid (CCl_3COOH).

Rules for writing formulas. Since compounds, like all matter, are electrically neutral, we must write formulas that have the proper number of negative and positive charges to balance one another. By following the simple rules that follow, you will be able to write the correct formulas for most of the compounds encountered in this course. We shall write the formula for aluminum oxide as our example.

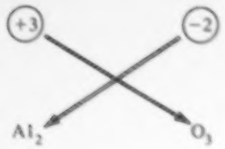
a. Write the chemical symbols (by convention the cation is written first).

Al O

b. Above each symbol, write the proper valence number (refer to periodic chart or list of radicals in the appendix).

$$\overset{+3}{Al}\quad\overset{-2}{O}$$

c. Obtain the proper subscripts by "crisscrossing" the numbers (drop all positive or negative signs).

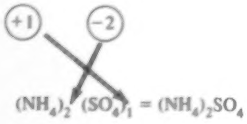


d. Now if the subscripts are (1) numerically equal or (2) equal to one, DROP THEM. *Example:*

$$Na_1Cl_1 = NaCl;\ Ca_2O_2 = CaO$$

e. If a radical is taken more than once, enclose it in parentheses and put the subscript *outside* the parentheses.

Example:



$$(NH_4)_2\ (SO_4)_1 = (NH_4)_2SO_4$$

f. Rewrite the formula, dropping the arrows and the valences that were written above. This is now the correct formula:

$$Al_2O_3$$

The neutrality of the formula may be checked by multiplying the valence of the cation times its subscript (which is the number of times it appears in one molecule). This will equal the total positive valence. Repeat this process for the anion to find the total negative valence. These two numbers must be equal in order to have a valid formula. From our example, we have:

$$\begin{array}{llrcrcr}
Al & : & +3 & \times & 2 & = & +6 \\
O & : & -2 & \times & 3 & = & -6 \\
\hline
\text{overall charge} & & & & & = & 0
\end{array}$$

Care must be taken in looking up the valences of metal ions because many of these can have more than one valence. To distinguish between the various valences, the suffixes *-ic* (higher valence) and *-ous* (lower valence) are added to the metal ion name. For example, cuprous chloride is CuC1, while cupric chloride is $CuCl_2$. Similarly, ferric (iron 3+) oxide is Fe_2O_3 and ferous (2+) oxide is merely FeO.

Exercises (042):

Write the formula for the following:

1. Calcium chloride.
2. Cupric iodide.
3. Ferric sulfate.
4. Barium hydroxide.
5. Mercuric nitrate.
6. Circle the tertiary compounds in the following formulas.

CaO　　$CaCl_2$　　$CaCO_3$　　Na_2SO_4

Complete the statement given below with each of the items that follow it, and in each case indicate whether the resulting sentence is true (T) or false (F).

A radical is a group of atoms

_____7. that are covalently bonded.

_____8. that are covalently bonded and that react as a single unit.

_____9. that have an electrical charge.

_____10. that may form new radicals by covalently bonding nitrogen to them.

043. Identify the types of chemical reactions that occur in given equations.

Chemical Reactions. You might compare chemical equations with the stenographer's shorthand script since they simplify and communicate an idea. It is not necessary that an equation contain complete information about a reaction. The more common reactions may be grouped according to the products they form, and some general statements may be made concerning these types of reactions.

Combination reaction. The first type of reaction that we will discuss is the combination reaction. The general equation representing combination reactions is: $A + B \longrightarrow AB$. A specific example of the combination reaction is the formula for a metal reacting with oxygen to yield a metallic oxide:

$$2Mg + O_2 \longrightarrow 2MgO.$$

Decomposition reaction. The general equation representing *decomposition reactions* is AB A + B. The following reactions are representative examples:

$$\begin{array}{lcl} CaCO_3 & \longrightarrow & CaO + CO_2 \\ Ca(OH)_2 & \longrightarrow & CaO + H_2O \\ 2KC10_3 & \longrightarrow & 2KC1 + 30_2 \\ H_2CO_3 & \longrightarrow & H_2O + CO_2 \\ 2HgO & \longrightarrow & 2Hg + O_2 \end{array}$$

Single displacement reaction. The general equation for a single displacement reaction is $A + BC \longrightarrow AC + B$. A more specific example is the reaction between zinc and copper sulfate:

$$Zn + CuSO_4 \longrightarrow ZnSO_4 + Cu$$

In this type of reaction, a more active metal replaces a less active one.

Double displacement reaction. The general equation for the double displacement reaction is $AB + CD \longrightarrow AD + CB$. A more specific example is the reaction that occurs between barium chloride and copper sulfate:

$$BaCl_2 + CuSO_4 \longrightarrow BaSO_4 + CuCl_2$$

This double displacement reaction is the most commonly occurring reaction. A double displacement reaction can be further subdivided into classes, of which the three most common are acid base, precipitation, and oxidation reduction.

Precipitation reaction. An acid reacts with a base to give a salt and water. An example is:

$$HCl + NaOH \longrightarrow NACl + H_2O$$

Precipitation is the throwing out of solution of a substance, usually a solid, as the result of some physical or chemical change having taken place.

In the precipitation reaction, two soluble substances in solution react to form one or more insoluble substances which precipitate from solution and settle out. This is indicated in an equation by an arrow pointing downward after the formula for the precipitated material.

$$BaCl_2 + Na_2SO_4 \longrightarrow BaSO_4 + 2NaCl$$

Oxidation-reduction reaction. You have probably always thought of oxidation as the phenomenon which takes place in the rusting of iron or the burning of combustibles, when a substance chemically combines with oxygen. Actually, oxidation includes any reaction in which an atom or ion loses electrons and, therefore, gains in positive valence. Reduction is the opposite, and results in a gain in electrons and a loss of positive valence. Oxidation and reduction occur simultaneously, never separately. Let's look at some of the possible valence states of sulfur, both in its elemental state and a compound. The valence of the sulfur is shown under the element or compound.

$$\begin{array}{ccccc} \longleftarrow\!\!\text{———} & \text{Oxidation} & & \text{Reduction} & \text{———}\!\!\longrightarrow \\ & SO_3 \quad SO_2 & & S \quad H_2S & \\ & +6 \quad\; +4 & & O \quad -2 & \end{array}$$

In the above illustration, the arrows point toward the direction of the reaction. Going from right to left (from H_2S toward SO_3), oxidation is occurring. Going from left to right (SO_3 toward H_2S), reduction is occurring. You can see that sulfur dioxide (SO_2), in going to sulfur trioxide (SO_3), presents a gain in valence or a loss of electrons, and is-according to our definition—oxidized. Going from elemental sulfur (S) to hydrogen sulfide (H_2S), there is a loss of valence or a gain in electrons, and the sulfur is reduced.

The processes of oxidation and reduction are reactions and can better be illustrated by using equations. We are considering oxidation reduction under double displacement reactions, even though single displacement reactions are all redox reactions. (Redox is a commonly used abbreviation for reduction oxidation.) Follow the equations below and see what happens during a single replacement reaction.

$$Zn + CuSO_4 \longrightarrow ZnSO_4 + Cu$$

Elemental zinc has no charge. In the copper sulfate molecule, copper has a valence if +2 and sulfate a valence of −2, thus giving the molecule electrical neutrality. Zinc sulfate in the product combines zinc with a valence of +2 and a sulfate with a valence of −2. The elemental copper formed has a valence state of 0, since it is elemental and neutral. This can be represented in the following manner so that the whole picture is clearly visible at one time:

$$Zn^{0} + CU^{+2}SO_4^{-2} \longrightarrow Zn^{+2}\,SO_4^{-2} + Cu^{0}$$

Zinc has gone from a valence state of 0 to a valence state of +2.

Exercises (043):

1. Indicate the type of reaction that occurs in each of the equations listed below by placing the appropriate letter in the space provided. Wach letter may be used once or more than once. The types of reaction are:

a. Combination reaction.
b. Precipitation reaction.
c. Double displacement reaction.
d. Single displacement reaction.
e. Decomposition reaction.
f. Acid-base reaction.

_____ (1) $HCl + NaHCO_3 \longrightarrow H_2CO_3 + NaCl$
_____ (2) $2Mg + O_2 \longrightarrow 2MgO$
_____ (3) $2KClO_3 \longrightarrow 2KCl + 3O_2$
_____ (4) $Zn + CuSO_4 \longrightarrow ZnSO_4 + Cu$
_____ (5) $BaCl_2 + CuSO_4 \longrightarrow BaSO_4 + CuCl_2$
_____ (6) $BaCl_2 + Na_2SO_4 \longrightarrow BaSO_4 + 2NaCl$

4-4. Writing Equations

Once the chemist knows the type of reaction that is taking place and is able to express this in symbolic terms, he or she is, in effect, writing an equation.

044. Explain the principles of balancing equations by citing reasons and ways an equation must balance and factors that affect equilibrium of a chemical reaction.

Reasons and Ways Equations Must Balance. Because of the basic fact that in a reaction matter is neither created nor destroyed, equations must balance. This is, the total number of atoms of each element on the left must equal the total number of atoms of each element on the right. Further, the net charge on the left must equal the net charge on the right. Practice in writing and balancing equations can be achieved by referring to practically any basic chemistry textbook.

Factors That Affect Chemical Equilibrium. Some chemical reactions are thought to be irreversible. For example, magnesium combines with oxygen to form an oxide, but the reverse of this reaction has not been observed. Other chemical reactions are reversible, either completely or incompletely. In the case of sulfur dixoide combining with water, the formation of H_2SO_3 and the reverse action occur simultaneously. This is indicated by a pair of double arrows:

$$SO_2 + H_2O \rightleftharpoons H_2SO_3$$

Certain factors affect the rate of a chemical reaction and disturb the equilibrium.

LeChatelier's principle. LeChatelier's principle states that a system in equilibrium will react to a stress by establishing a new equilibrium. Some of the factors that may affect the equilibrium are light, pressure, temperature, and catalysts. The addition of heat will speed up a reaction only if the reaction is endothermic; that is, it absorbs heat. In other words, the equilibrium of a reaction will be shifted in the direction which absorbs heat if heat is applied.

Light. Some reactions are accelerated by the addition of light energy. It is for this reason that reagents are kept in brown bottles. The addition of pressure applies to gases, based on a corresponding volume change in the reactants or compounds formed.

Catalysts. The effect of catalysts is of greater interest to the clinical chemist than many other factors that affect a chemical reaction. A *catalyst* is a substance that speeds up or slows down a chemical reaction without being changed itself. Enzymes all fall into the category of biocatalysts, or catalysts that activate or accelerate biological processes.

Concentration. In addition to the factors mentioned, the concentration of the reacting substances influences the rate of a chemical reaction. Upon reaching equilibrium, if the concentrations of the substances produced are multiplied together and divided by the product of the initial or unreacted substances, the expression is termed the "equilibrium constant." When the equilibrium constant is applied to an ionic equilibrium, it becomes the ionization constant. The value of this constant or ratio will depend upon the nature of the reacting substances, but it will be independent of the various concentrations. Besides ionization constants there may also be expressions of solubility product constants, hydrolysis constants, and other constants. When two reactants, *A* and *B*, in a reversible reaction form products *C* and *D*, the equilibrium constant is expressed mathematically as follows:

$$\frac{[C]\ [D]}{[A]\ [B]} = K$$

The expressions in brackets represent molar concentrations of the reactants and products of the reaction. Although values of these expressions may vary, the relationship remains constant. The value of using such an equilibrium constant to calculate molar concentrations is apparent.

Exercises (044):

1. Why must a chemical equation balance?

2. In what two ways must a chemical equation balance?

3. List four factors that affect chemical equilibrium.

4. Briefly state LaChatelier's principle.

5. Application of heat to a system that is in equilibrium causes the equilibrium to shift in what direction?

6. Why are reagents stored in brown bottles?

7. Pressure changes the equilibrium only in case of __________.

8. What is a catalyst?

9. What type of catalyst is of most interest to the clinical chemist?

10. Briefly state the meaning of the following formula:

$$\frac{[C]\;[D]}{[A]\;[B} = K$$

11. The equilibrium constant is used to calculate __________.

4-5. Acids, Bases, and Salts

Acids are often defined as substances containing hydrogen that donate hydrogen ions in solution. Bases are defined as substances containing hydroxide groups that donate hydroxide ions in solution. Salts are the results of reactions between an acid and a base. In other terms, salts can be defined as a compound formed by the combination of a substance with a negative ion other than OH^- or with any positive ion other than H^+.

045. Identify acids, bases, and salts, their properties; and terms used to describe their chemical reactions.

Acids. An acid is any substance that furnishes hydrogen ions in solution to a concentration greater than 1×10^{-7} moles per liter. A mole is defined as a gram of molecular weight. (We shall discuss this area in greater detail in another chapter.) An acid is said to be strong if it is highly *dissociated, and weak if it is only slightly dissociated.* By dissociation we mean the phenomenon whereby atoms of a compound separate from each other as ions in solution. The Bronsted-Lowry concept emphasizes the nature of the solvent and defines an acid as a substance that can increase the hydrogen ion concentration, rather than restricting the definition to compounds that contain hydrogen ions. This broaded definition includes substances that react with water to produce hydrogen. An example is carbon dioxide, as shown in the following equation:

$$CO_2 + H_2O \longrightarrow H^+ + HCO_3^-$$

Another example is aluminum chloride, which furnishes hydrogen ions, as follows:

$$AlCl_3 + 3H_2O \longrightarrow 3H^+ + 3Cl^- + AlOH_3$$

Except in the context of the Bronsted-Lowry definition, aluminum chloride is not usually considered an acid.

Acids have the following common properties: they usually have a sour taste, affect some indicators, neutralize bases to form salts, react with some metals to form salts, react with some metallic oxides to form salts, react with carbonates to form carbon dioxide and water, and are usually (but not always) soluble in water. Generally, acids may be prepared by the following methods:

a. Reacting water with a nonmetallic oxide:

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

b. Reacting sulfuric acid with the salt of the acid:

$$H_2SO_4 + NaCl \longrightarrow NaHSO_4 + HCl$$

c. Direct combination:

$$H_2 + Cl_2 \longrightarrow 2HCl$$

Acids may be classified in accordance with the number of hydrogen ions (protons) furnished by each molecule.

Monobasic acids give one proton or hydrogen ion per molecule. Examples are HCl and HNO_3. Tribasic acids have three protons or hydrogen ions per molecule. Examples: H_3PO_4, H_3BO_4. The term "polyprotic" pertains to acids that furnish more than one proton per molecule.

Bases. A base is a substance containing the hydroxyl (OH) group which, when dissolved in water, forms no negative ions other than OH^- ions. Bases are proton acceptors. Sodium hydroxide ionizes in water solution to Na^+ and OH^-. The only negative ion is the hydroxyl ion. When acids and bases react in a reaction called neutralization, the proton (H^+ ion) reacts with the proton acceptor (OH^- ion) to form water. Bases have the following common properties: They have a bitter taste in solution, feel slick and slippery like soap, affect some indicators, react with acids to produce salts, and react with nonmetallic oxides to form salts. Generally bases may be prepared by the following methods:

a. Some active metals react with water to form bases:

$$2Na + 2HOH \longrightarrow 2NaOH + H_2$$

b. Some metallic oxides react with water to form bases:

$$CaO + HOH \longrightarrow Ca(OH)_2$$

c. When salts of calcium, magnesium, and iron come in contact with the soluble bases, double displacement reactions occur, forming insoluble bases which precipitate:

$$FeCl_3 + 3NaOH \longrightarrow Fe(OH)_3 + 3NaCl$$

d. Some substances do not have an OH group but act as bases. For example, ammonia reacts with water as follows:

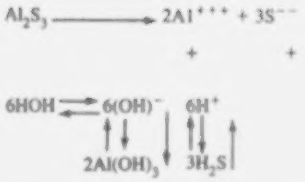
$$NH_3 + H_2O \longrightarrow NH_4^+ + (OH)^-$$

Ammonia is considered a base because it furnishes hydroxyl ions. Actually, once the ammonia has reacted with water, it is no longer ammonia.

Salts. In very brief terms, a salt is formed by the combination of any negative ion except $(OH)^-$ with any positive ion except $(H)^+$. For example:

$$NaOH + HCl \longrightarrow NaCl + H_2O$$

Sodium chloride is, of course, a salt. Some salts are capable of incorporating and splitting water by hydrolysis. Generally, salts formed from strong acids and strong bases do not hydrolyze, but those formed from weak acids and weak bases do hydrolyze. For example, aluminum sulfide, formed from a very weak acid and a very weak base, hydrolyzes in water solutions as follows:


$Al_2S_3 \longrightarrow 2Al^{+++} + 3S^{--}$
+
+
$6HOH \rightleftharpoons 6(OH)^-$
$6H^+$
$2Al(OH)_3$
$3H_2S$

This hydrolysis goes to completion, because the hydrogen sulfide that is formed bubbles off and prevents the reverse reaction from occurring by removing one of the reactants. Thus, equilibrium is not established as would otherwise be expected.

Composition determines whether the particular salt is acid, alkaline, or neutral. Many of the salts are white crystalline substances, but the salts of certain metals are brightly colored.

Exercises (045):

1. Match each term in column B relating to acids, bases, and salts with its appropriate definition or description in column A.

Column A

_____ (1) Furnishes hydrogen ions.
_____ (2) Defines an acid as a substance that can increase the hydrogen ion concentration.
_____ (3) Example of a monobasic acid.
_____ (4) Example of a tribasic acid.
_____ (5) Acid that furnishes more than one proton per molecule.
_____ (6) Substance containing hydroxyl (OH) group, which, when dissolved in water, forms no negative ions other than OH^- ions.
_____ (7) Acid and bases react in which the proton (H^+ ion) reacts with the proton acceptor (OH^- ion) to form water.
_____ (8) Compound resulting from the combination of any negative ion except $(OH)^-$ and with any positive ion except $(H)^+$.
_____ (9) Salts formed from strong acids and strong bases.
_____ (10) React with carbonates to form carbon dioxide and water.
_____ (11) React with nonmetallic oxides to form water.

Column B

a. Acid.
b. Bronsted-Lowry definition of an acid.
c. Polyprotic.
d. Base.
e. Neutralization.
f. Do not hydrolyze.
g. Salt.
h. HNO_3.
i. H_3PO_4.
j. Property of an acid.
k. Property of a base.
l. Hydrolysis takes place.

Column A

_____ (12) Salts formed from weak acids and weak bases.

4-6. Organic Compounds

Organic chemistry is the study of the compounds of carbon—compounds which are vital in the composition and function of living things. Organic substances may occur naturally, they may be prepared by synthesis, or they may be semisynthetic—as is true of the antibiotics. Carbon possesses the unique ability to link together (bond) to form complex molecules.

These molecules may form continuous chains, branching chains, closed chains, or closed chains with separating elements other than carbon. Figure 4-11 illustrates some of these chains. Bonds join the atoms together and determine the reactions into which the compound will be able to enter. The structure of organic compounds and the bonding they possess is best shown by graphic formulas.

046. Identify given organic compounds by matching each with its appropriate composition, incidence, description, or use.

Hydrocarbons. Compounds composed solely of carbon and hydrogen are called *hydrocarbons*, and can be either straight chained or cyclic. In the straight-chain group are several homologous series—that is, series of compounds differing from member to member by a common increment. By substituting an element such as chlorine for one or more hydrogen atoms in a hydrocarbon, hydrocarbon derivatives are achieved. Carbon tetrachloride, chloroform, and iodoform are important examples of derived hydrocarbons.

Alcohol and Ether. Aliphatic or open-chain *alcohols* consist chemically of two distinct parts: a hydrocarbon chain, often represented by the letter "R," and one or more OH groups. Unlike the OH group inorganic compounds, the OH of alcohols is not a basic or alkaline group. Ethyl alcohol, isopropanol, glycerin, and propylene glycol are examples of alcohols. *Ethers* are dehydration products of two molecules of alcohol and may be either simple or moxed. Diethyl ether is a simple ether widely used as a general inhalation anesthetic, and is composed of two ethyl groups, as shown in figure 4-12.

Aldehydes and Ketones. *Aldehydes* are oxidation products of primary alcohols and are easily recognized by the carbonyl group and hydrogen attached to the alkyl radical. Aldehydes are usually odoriferous, ranging from very disagreeable to fragrant. Formaldehyde, paraldehyde, and chloral hydrate are examples of aldehydes. *Ketones* are odication products resulting from secondary alcohols and resemble aldehydes in structure except that they have an additional alkyl radical in place of the hydrogen on the aldehyde. Acetone is a ketone; it is illustrated in figure 4-13.

Esters. Esters are products formed from the reaction between an alcohol and an acid, an acid chloride, or an acid anhydride. Cholesterol esters may be assayed in the clinical laboratory and other esters may be used as reagents. *Fats* and fixed oils are glycerol in their structure. They are generally hard, have high melting points, and are not as greasy as fats.

Sterols and Phenols. *Sterols* are high molecular weight cyclic alcohols derived from a portion of fats. Many hormones have a structure characteristic of sterols. Cyclic structures have three or more atoms joined into a closed ring. Benzene, toluene, xylene, napthalene, and phenanthrene are basic cyclic structures. When a hydroxyl group is substituted onto an aromatic ring, the compound formed is not an alcohol, but a *phenol*. Phenol, resorcinol,

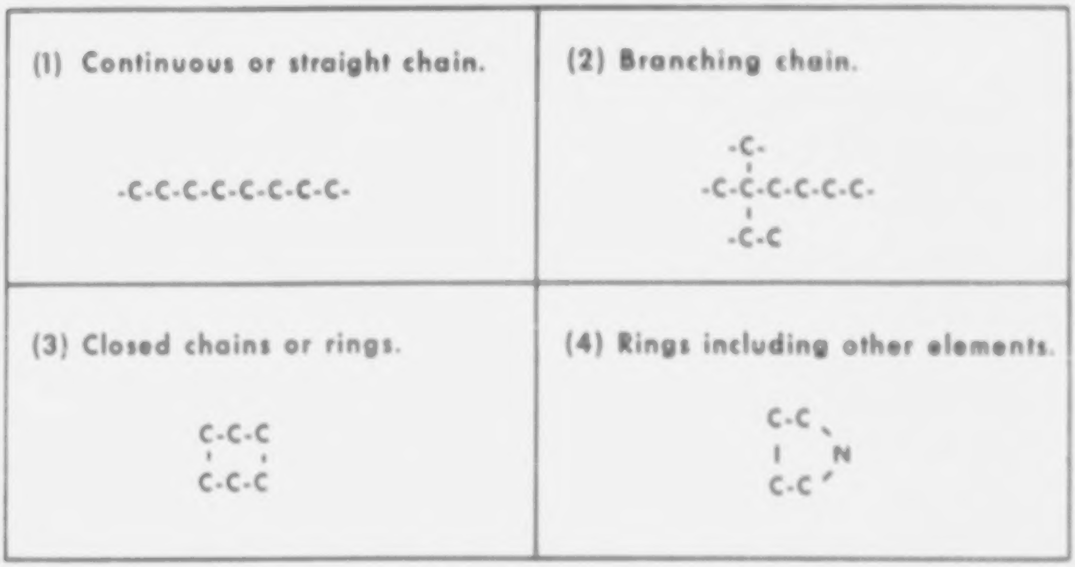

(1) Continuous or straight chain.
-C-C-C-C-C-C-C-C-
(2) Branching chain.
-C-
-C-C-C-C-C-C-
-C-C
(3) Closed chains or rings.
C-C-C
C-C-C
(4) Rings including other elements.
C-C
N
C-C

Figure 4-11. Types of carbon chains.

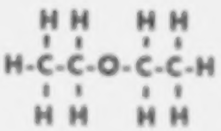


Figure 4-12. Structure of diethylether.

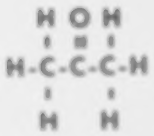


Figure 4-13. Structural formula for acetone.

thymol, and cresol are significant phenols. Aromatic acids contain an aromatic ring and a carboxyl group. They are important mainly because of the analgesics, antispasmodics, antiseptics, and local anesthetics produced by their esterification. Aromatic halogenated compounds, such as benzene hexachloride, DDT, halazone, merbromin, isodine, diiodohydroxyquin, and iodochlorhydroxyquin are generally antiseptic in nature. A few have insecticidal ability.

Amines and Amides. *Amines* and *amides*, the nitrogen-containing compounds, are very important in medicine and pharmacy because into these classes fall the alkaloids, antihistamines, sulfa drugs, barbituates, analgesics, and local anesthetics. *Amino acids* are difunctional organic compounds containing an amino group and an acid group, either carboxyl or sulfonic. Approximately 30 amino acids are known to man. When two or more amino acids condense, eliminating a molecule of water, a peptide is formed.

Proteins, Carbohydrates, and Glycosides. *Proteins* are polymers of amino acids. They are an important source of food for the body and, on digestion, break down into amino acids that can be absorbed into the blood stream. *Carbohydrates* are polyhydroxy aldehydes, ketones, or organic compounds that yield these substances on hydrolysis. They are definite chemical compounds, not merely hydrated forms of carbon. Monosaccharides contain three or more carbons and cannot be hydrolyzed. Disaccharides are made up of two molecules of monosaccharides and, upon hydrolysis, separate into the two monosaccharides of which they are composed. Polysaccharides are more complex carbohydrates. Dextrose and fructose are monosaccharides; sucrose and lactose are disaccharides; and starch is an example of a polysaccharide. *Glycosides* are organic compounds consisting of sugars with hydroxy-containing molecules.

Steroids. Steroids are a class of compounds found in both plants and animals. This class includes sterols and several hormones covered in Volume 3, as well as bile acids, covered in Volume 2.

Refer to any standard textbook of organic chemistry for a complete review of the subject and for structural formulas. We shall discuss some of the organic substances, such as carbohydrates and proteins, in some detail in Volume 2.

Exercises (046):

1. Match each organic compound in column B with its description in column A. Each element in column B may be used once or more than once.

Column A

_____ (1) Found in both plants and animals; includes sterols.
_____ (2) Polymers of amino acids.
_____ (3) Consisting of a combination of sugars with hydroxy-containing molecules.
_____ (4) Formaldehyde, paraldehyde, and chloral hydrate.
_____ (5) A hydrocarbon chain, often represented by the letter "R," and one or more OH groups.
_____ (6) Carbon tetrachloride, chloroform, and iodoform.
_____ (7) High molecular weight cyclic alcohols, such as benzene, toluene, xylene, naphthalene, and phenanthrene.
_____ (8) Nitrogen-containing compounds important in medicine and pharmacy.
_____ (9) Formed from the reaction between an alcohol and an acid, an acid chloride, or an acid anhydride.
_____ (10) Formed when a hydroxyl group is substituted onto an aromatic ring.

Column B

a. Hydrocarbons.
b. Esters.
c. Aldehydes.
d. Aliphatic or open-chain alcohols.
e. Amines and amides.
f. Sterols.
g. Proteins.
h. Carbohydrates.
i. Steroids.
j. Glycosides.
k. Phenol.

Solutions

THE IMPORTANCE of pure, accurate solutions cannot be overemphasized in a chemistry laboratory. In fact, the accuracy of all results obtained by a laboratory depends upon reagents that you may prepare.

Quality solutions are not the result of magic. They are the culmination of intelligent, exacting application of basic principles that you can learn. You must know the differences in the various percent solutions prepared in the laboratory. You must precisely define molar concentration, normality, equivalent weights, and milliequivalent weights. You should be able to explain pH and to standardize acids and bases. What is the concept of a buffer system, and how does an indicator function? The answers to these and other questions are found in the basic principles of solutions set forth in this chapter.

5-1. Nature of Solution

As pointed out earlier, chemical reactions involve the interaction of atoms. Any mixture in which the components are present as individual atoms or molecules is considered a solution. You can see then that as a chemistry technician you will be working with solutions. This is a very necessary and important requirement in the chemistry laboratory.

047. Identify the terminology associated with solutions, the basic nature of solutions, and factors that determine solubility.

Terminology. In practical terms, a *solution* is a homogeneous mixture of two or more substances. This homogeneous condition distinguishes a solution from a suspension or dispersion in which the mixture is not the same throughout. Even though some substances dissociate into their respective ions, the result is still a mixture, and solutions may be considered as such. The dissolving medium is termed the *solvent*, and the substance being dissolved is termed the *solute*. Since matter may exist as a solid, liquid, or gas, any combination of the three states of matter can be thought of as a solution if it fits the basic definition. Most of the solutions used in a clinical laboratory are solids in liquids or liquids in liquids. If a solid such as iodine diffuses into another solid, this combination may also qualify as an example of a solution. Gases can also be dissolved in a liquid to make up a solution. This particular type of solution frequently has a physiological application. It may not be readily apparent which constituent is the solute and which is the solvent—as, for example, in the case of two liquids. It has been generally held by convention that if the solution is aqueous, water is the solvent. If water is not present, the liquid present in the larger quantity is the solvent.

Types. There are various ways to classify solutions. One way is in terms of concentration. The amount of solute present in a given volume of solution is an expression of concentration. There are four major factors that determine how much solute will dissolve in a given volume of solution. These factors are (1) the nature of the solute, (2) the nature of the solvent, (3) the temperature, and (4) (for gases only) the pressure. In this discussion we shall not refer to pressure, since it applies only to gases. Since the nature of both solute and solvent is determined by the requirements of the problem, the common variable is that of temperature. For most purposes, we can say that at any given temperature a solution is *saturated* if it holds all of the solute it can normally contain at that temperature. The reason for inserting the word "normally" is that it is possible to use a technique which results in a supersaturated solution—that is, a solution that contains more solute than it can normally contain at that temperature. One such technique would be to heat the solution and cool it again. If the solution is undisturbed, the excess solute remaining in solution causes the solution to be supersaturated. The addition of a "seed crystal" or a scratch on the inside of the container may cause the excess solute to precipitate from solution. Heating a solution does not always increase the solubility of a solute. Sodium chloride, for example, is only slightly more soluble in hot water than it is in cold water. Finally, in describing concentration, the chemist refers to an *unsaturated solution*. This is obviously a solution that does not contain all of the solute it can normally contain at a particular temperature.

Exercises (047):

1. Match the terms in column B with the definitions or descriptive statements in column A.

Column A

_____(1) Homogeneous mixture of two or more substances.
_____(2) Mixture in which the substances are not uniformly suspended or dispersed throughout.
_____(3) Medium in which another substance is dissolved.
_____(4) Substance dissolved.
_____(5) The solvent in an aqueous solution.
_____(6) The amount of solute present in a given volume of solution.
_____(7) Factors that determine how much solute will dissolve in a given volume of solution.
_____(8) Common variable in solutions.
_____(9) A solution that holds all of the solute that it normally can at any given temperature.
_____(10) Solution that contains more solute than it normally can at a given temperature.
_____(11) May cause excess solute to precipitate in a supersaturated solution.
_____(12) Factor that usually causes more solute to dissolve, but not always.
_____(13) Solution that does not contain all of the solute that it can normally contain at a particular temperature.

Column B

a. Supersaturated solution.
b. Solute.
c. Nature of solute, nature of solvent, temperature, and pressure (for gases).
d. Heating.
e. Solution.
f. Suspension or dispersion.
g. Unsaturated solution.
h. Seed crystal or scratch on inside of container.
i. Water.
j. Temperature.
k. Concentration.
l. Saturated solution.
m. Solvent.

5-2. Expressions of Solution Concentration

Quantitative chemical analysis requires a variety of solutions of known concentration. How accurate these solutions must be depends upon how they are to be used. For example, a percent solution of sodium hydroxide is adequate to adjust the pH of a urine specimen, but a very precise normality of the chemical is required to standardize an acid. In this section, we shall detail the various solution concentrations used in clinical chemistry.

048. Determine the correct quantity of solute or solvent required to prepare percent solutions of varying concentrations.

Percent Solutions. In speaking of percent solutions, you are merely expressing parts of *solute* per hundred parts of *final solution*. Obviously, the term "parts" is not specific enough. It may be expressed in weight (as grams, mg, etc.) or in volume (as liters, ml, etc.). Therefore, it is necessary to specify which of four possible conditions exist. The four possibilities are (1) weight in volume (W/V), (2) volume in volume (V/V), (3) volume in weight (V/W), and (4) weight in weight (W/W). In clinical work, weight in volume (W/V) and volume in volume (V/V) are the most common. It is generally assumed that a percent solution means a solid in a liquid if the solute occurs as a solid in its natural state. Likewise, if the solute occurs as a liquid, volume in volume is implied. This assumption, though widely applied, is something less than accurate. Acids, for example, are most often liquids, but their concentrations are seldom expressed on the basis of volume in volume. However, if percent is used in conjunction with liquid reagents, including acids, it must be considered volumetric (V/V), unless otherwise specified.

Preparing V/V Solutions. By definition, concentration is expressed in terms of final volume of solution, not in terms of the amount of solvent. A 10 percent V/V solution is prepared by taking 10 ml of solute and adding enough solvent to bring the final volume to 100 ml (termed q.s. to volume). Any solution proportional to this would also be 10 percent. For example, there could be 1 ml in a final volume of 10 ml, or 100 ml in a total volume of 1000 ml. You need only to set up a single ratio and proportion to prepare any other amount of a 10 percent solution. Since volume varies directly with temperature, a solution should be brought to room temperature before the final volumetric adjustment is made, provided such accuracy is required. It is interesting, though not practically significant in most situations, that miscibility—or in other words, intermingling of molecules—of two liquids may result in a final total volume which is less than the sum of the individual volumes. In diluting a solution to a final volume, this phenomenon is ignored. In pharmaceutical techniques it is understood that 50 percent ethanol means 50 ml of ethanol and 50 ml of diluent (solvent), rather than 50 ml of ethanol q.s. to 100 ml. Such accuracy is not generally required in the medical laboratory, though it is true that the total volume in this example is a few milliliters less than 100 ml because of molecular spacing.

Preparing W/V Solutions. In the case of dry reagents, it is a relatively simple matter to weigh a reagent and dissolve it in a suitable solvent. When a high degree of accuracy is desired, a reagent may be desiccated and weighed with an analytical balance which can approach an accuracy of 2 mg without technical difficulty. First, you should decide the degree of accuracy required; and second, you should prepare large enough quantities to minimize error and achieve the accuracy required. You should also be cautioned that some reagents such as picric acid cannot be dehydrated without danger of explosion. The matter of expressing units of weight per 100 parts of solution is, then no problem. Of somewhat greater difficulty is the need to express liquid reagents in terms of weight. The most direct approach is to divide the grams needed by the weight of 1 ml (that is, specific gravity) to convert weight to volume. Obviously, it is easier to measure liquid by volume than to weigh it. Another aspect you must consider when determining the weight of liquid is its assay value. All reagents are not 100 percent pure. To correct for this lack of purity, you need to refer to the manufacturer's labeling and then use this assay value as a correction factor. For example, if the assay is 50 percent for a particular reagent, it will be necessary to use twice as much as you would have used if it had been 100 percent pure. In other words, it will be necessary to multiply by 100/50, or 2. In summary, a formula that can be stated as follows is used:

$$\frac{\text{Grams needed}}{\text{sp gr}} \times \frac{100}{(\%\ \text{purity})} = \text{ml of liquid reagent use}$$

Percent purity is expressed in the equation as a percent figure, not as a decimal.

Example:

How many milliliters of concentrated acetic acid (sp gr 1.049 and 99.5 percent purity) are needed to prepare 250 ml of a 10-percent (W/V) solution?

Answer:

You may use formula #1, Summary of General Formulae, in Appendix G (use this formula as necessary to solve problems on solutions).

Grams of solute required equals:

$$\frac{(\%\ \text{W/V}) \times (\text{volume in ml of final solution})}{100}$$

$$\frac{10 \times 250}{100} = 25\ \text{gm}$$

Therefore:

$$\frac{25}{1.049} \times \frac{100}{99.5} = 24\ \text{ml of acetic (a liquid) is needed}$$

You may note that this type of problem is quite different from one involving simple percentage calculated on a volume basis, as described earlier in this section.

Exercises (048):

1. How many milliliters of acid are used to make 100 ml of a 10 percent V/V solution?

2. How many milliliters of a base are used to make 500 ml of a 15 percent V/V solution?

3. How would you prepare a 50 percent ethanol V/V solution?

4. How would you prepare 3000 ml of 0.85 percent NaCl W/V solution?

5-3. Molar Solutions

When we combine two solutions in a test tube, the nature of the reaction that occurs depends upon the molarity of both solutions—that is, the ratio of the dissolved substance or solute to the liquid in which it is dissolved, or solvent. This is because chemicals react on a molecule-by-molecule basis. In your duties as laboratory technician, you will frequently observe the use of molar solutions in chemical analyses. This section provides you with the knowledge that you will need to understand and prepare these solutions, when necessary.

049. Determine the correct quantity of solvent or solute required to prepare molar solutions of specified concentrations.

Definition. A molar solution is one that contains one mole (1 gram molecular weight) of the solute in one liter (L) of solution. The symbol for molarity is *M*.

It has been found experimentally that there are 6.023×10^{23} molecules in a gram molecule or mole, so the actual number of molecules in a gram molecular weight is $6.023 \text{ X } 10^{23}$. This number is an important constant in physical chemistry and is known as the Avogadro constant or Avogadro number.

When we make a 1-molar solution of any substance, we are dissolving the same number (6.02×10^{23}) of *molecules* in a volume of solvent. The sum of the assigned weights of each atom in a molecule represents the molecular weight, or gram molecular weight if expressed in grams. The gram molecular weight of any substance in 1 L solution represents a 1-molar solution; or, we say the solution has a molarity of 1.

For example, a 1-molar solution of copper sulfate ($CuSO_4$) contains 159.6 g per liter. Occasionally, reagents contain water of crystallization, and for every molecule of the pure compound there are one or more molecules of water. To expand the example used, copper sulphate may be procured as hydrous copper sulfate ($CuSO_4 \cdot 5H_2O$).

(The label on the bottle gives this information.) If you use $CuSO_4 \cdot 5H_2O$ instead of anhydrous reagent ($CuSO_4$), it will be necessary for you to weigh out one formula weight, or 249.6 g, in order to achieve a 1-molar solution. The reason for this is that 5 molecules of water for every molecule of copper sulphate contributes nothing to the number of copper ions or sulfate radicals present, but water does contribute to the weight of the reagent. Thus, we see that either 159.6 g of $CuSO_4$ or 249.6 g of $CuSO_4 \cdot 5H_2O$) will make a 1-molar solution of $CuSO_4$ if expressed as solute in 1 L of final solution.

Equivalent Weight. The concept of molarity is based on the weight of the molecule under consideration and says nothing of the combining capacity of the substance. A more useful concept is normality, which is based on equivalent weight. An equivalent weight (or gram equivalent weight, if expressed in grams) is the molecular weight divided by the total positive *or* negative valence. By the total positive valence, we mean the number of replaceable hydrogen ions (or cations); by total negative valence, we mean the number of replaceable hydroxyl groups (or anions). For example, a reaction involving the hydrogen ions in sulfuric acid (H_2SO_4) would require that we divide by 2, since there are 2 hydrogen ions per molecule. We may also speak of combining weight or equivalent weight of *an element*, which is the atomic weight divided by the valence. Relating this concept to the Avogadro number, the combining weight either picks up or releases 6.0235×10^{23} number of electrons. If, as an example, aluminum with a valence of +3 reacts with oxygen, each atom of aluminum will lose 3 electrons. In a 1-molar solution, 3 times the Avogadro number of electrons will be released. Thus, the weight of aluminum that would release one-third this number of electrons is one-third the gram atomic weight.

Keep in mind that equivalent weight is always expressed with respect to one of the ions or radicals. In the case of a solute which furnishes only two kinds of ions, the number of positive ions equals the number of negative ions. If more than two kinds of ions are furnished, it is important that it be specified which ion is being referred to, based on the particular chemical reaction. For example, disodium ammonium phosphate ($Na_2NH_4PO_4$) dissociates to furnish 2 sodium ions, 1 ammonium ion, and 1 phosphate radical. Thus, the equivalent weight with respect to the sodium ion is one-half the molecular weight. In the case of the ammonium ion, the equivalent weight is the same as the molecular weight of the salt because NH_4 has a valence of +1.

In spite of this obvious situation, of which double decomposition is an example, you may be inclined to believe that equivalent weight (and hence normality) has no relationship to the nature of the reaction. Just the opposite is true; equivalent weight is a concept which takes into account the combining capacity with reference to a particular ion. As a final example, we might consider the equivalent weight of ferrous sulphate $FeSO_4$. In oxidation-reduction reactions, its equivalent weight is the same as its molecular weight. In double decomposition reactions the equivalent weight is one-half this amount.

How significant is this rather confusing point to you as a medical laboratory technician? Ordinarily, you are dealing with acids and bases wherein it is obvious that only two kinds of ions are furnished. But you need to be aware of equivalent weight as an expression of combining weight for purposes of the particular reaction in which the chemical is to be employed.

Normal Solutions. Just as 1 g molecular weight per liter represents a 1-molar solution, so does 1 g equivalent weight per liter represent 1-*normal* solution. Normality is expressed by a capital N. In common usage N/2 means one-half normal, N/10 means one-tenth normal, etc. The reason for using the concept of normality is already apparent to you. A 1-molar solution of sulfuric acid (H_2SO_4) contains 2 atoms of hydrogen per molecule, and will furnish twice as many hydrogen ions as will hydrochloric acid (HCl). Hence, a 1-molar solution of sulfuric acid represents a 2-normal solution, whereas a 1-molar solution of HCl is also a 1-normal solution. Do you see why?

Example:

How many milliliters of phosphoric acid (H_3PO_4 are needed to prepare a 1-normal solution, using phosphoric acid reagent of specific gravity 1.8 and assay value of 80 percent?

Answer:

$$\text{Grams needed} = \frac{\text{molecular wt.}}{\text{valence}} = \frac{98}{3} \text{ or } 32.7 \text{ gm}$$

Using the formula given in percent solutions, we find $\frac{32.7}{1.8} \times \frac{100}{80}$ would give the milliliters of phosphoric acid to be diluted to a volume of 1 L for a 1-normal solution. As previously stated, the term "valence" in the denominator is synonymous with the number of acidic or ionizable hydrogen ions per molecule.

The following formulas from Appendix G may be used.

$$\text{Ml concentrate required} = \frac{\text{mol wt X desired normality X final vol}}{\text{valence X 1000}}$$

or

$$\frac{\text{Normality desired X gram equivalent weight}}{\text{specific gravity}} \times \frac{100}{\text{percent purity}}$$

$$= \text{milliliters for 1 L of desired normality solution}$$

Exercises (049):

1. Problem Situation No 1: Your supervisor asks you to prepare 500 ml of a 2M solution of NaOH. How many

grams of 100 percent chemically pure (CP) NaOH would be necessary?

2. Problem Situation No 2: Your supervisor gives you an assignment to prepare 1 L of a 2N solution of H_2SO_4. You find a bottle of sulfuric acid with a specific gravity of 1.834 and 98.0 percent purity. How would you prepare this solution?

3. How many equivalent weights are in a 1 M solution of H_3PO_4?

4. How many grams of sulfuric acid (H_2SO_4) are needed to prepare 200 ml of a 1N solution?

5. How many grams of HCl are necessary to prepare 200 ml of 0.001N HCl solution? (NOTE: Approximately 2.30 ml of HCl is equivalent to 1 gm of pure solute.)

6. How many milliliters of concentrated HNO_3, specific gravity 1.4 and 70 percent pure, are necessary to prepare 500 ml of 0.1M HNO_3?

7. How many milliliters of concentrated sulfuric acid, specific gravity 1.84 and 95 percent pure, are necessary to prepare 100 ml of a 2M solution?

8. How many milliliters of concentrated HCl having a specific gravity of 1.19 and 38 percent pure are necessary to prepare 500 ml of 1.5N HCl?

5-4. Preparation of Solutions by Dilutions

In many instances it is best economy for the laboratory to purchase concentrated solutions and dilute as necessary to perform tests. It is also economical and convenient for the laboratory to prepare concentrate solutions from basic ingredients and dilute them as needed. You should be able to use concentrated stock solutions, where possible, to save time, money, and storage space.

050. Use the volume-concentration (V–C) formula to solve problems concerning the amount of basic ingredients to use in preparing a dilute solution of a given concentration.

V–C Formula. Whenever it is necessary to prepare a dilute solution from a more concentrated one, the following formula holds true:

$$V_1C_1 = V_2C_2$$

Where V_1 is the volume of the solution to be diluted, C_1 is the concentration of the solution to be diluted, V_2 is the volume of the diluted solution, and C_2 is the concentration of the diluted solution.

As suggested in the sections 5–1 and 5–2, concentration times volume is a means of expressing the amount of solute present. For example, in 100 ml of a 10-percent W/V solution, there are 10 gm of solute. Diluting this solution merely increases the volume and proportionally decreases the concentration. The *amount of solute does not change*. If the volume were increased to 1 L, the concentration would be decreased to 1 percent, since the amount of solute is still 10 gm. It should be noted that this formula holds true, regardless of the terms used to express concentration, so long as C_1 and C_2 concentrations are expressed in the same terms (mg/ml, g/L, etc.).

Using the V–C Formula. The next point involves understanding how to use the V–C formula. Concentration can be expressed in any terms, including those of percent

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and normality. With this in mind, let us look at some more example problems.

Example:

How many milliliters of 100 percent ethanol (absolute alcohol) are required to prepare 800 ml of 30 percent?

Answer:

Fill in the values as follows:

$V_1 = ?$
$C_1 = 100\%$
$V_2 = 800 \text{ ml}$
$C_2 = 30\%$

Then:

$$V_1 = \frac{800\ (30)}{100} = 240 \text{ ml}$$

Example:

If 200 ml of 0.1N sulfuric acid are diluted to 1 L, what is the resulting normality?

Answer:

$V_1C_1 = V_2C_2$
$V_1 = 200 \text{ ml}$
$C_1 = 0.1\text{N}$
$V_2 = 1000 \text{ ml}$
$C_2 = ?$
$(200)(0.1) = (1000)C_2$
$0.02 = C_2$

Exercises (050):

1. How many milliliters of 85 percent alcohol are required to prepare 100 ml of 65 percent alcohol?

2. How many milliliters of 3 percent sugar solution can be made from 600 ml of 15 percent sugar solution?

5-5. Milliequivalent Weight

When working with very dilute solutions, it is desirable to express concentration in units that are equal to each other. Just as the gram is divisible into 1000 milligrams and the liter into 1000 milliliters, the equivalent is divisible into 1000 units so that

$$1 \text{ Equivalent (Eq)} = 1000 \text{ milliequivalent}$$

or

$$0.001 \text{ Eq} = 1 \text{ mEq}$$

051. Solve problems to determine the milliequivalent per liter and millimole of a given substance.

Equivalent Weight. You know from previous sections dealing with equivalent weight and normality that equivalents are related to chemical reactivity in a definite proportion. In fact, one chemical equivalent has the same chemical reactivity as any other chemical equivalent.

Formula Weight. In physiological chemistry, this concept is useful for calculations involving electolytes and acid-base balance. When expressing the concentration of body electrolytes and in dealing with dilute solution, the clinical worker uses milliequivalent weights (mEqwt) rather than equivalent weights. The concentrations of electrolytes in body fluid are normally quite low so that mEq/L is a practical unit. An mEq weight is 1/1000 of the equivalent weight or is equal to the millimolecular weight divided by the valence. In the case of sodium, the molecular weight is 23; the equivalent weight is also 23 because

$$\frac{\text{Molecular weight}}{\text{valance}} = \text{equivalent weight}$$

$$\frac{23}{1} = 23$$

The mEq weight is also 23. The equivalent weight and mEq weight have the same numerical designation because equivalents relate to reactivity or combining capacity rather than to weight alone. This can be more completely understood by converting a weight term, 310mg/dl of sodium, to mEq/L. The following formula will make this conversion:

$$\frac{\text{mg/dl} \times 10}{\text{mEq wt}} \text{ or } \frac{\text{mg/L}}{\text{mEq wt}} = \text{mEq/L}$$

$$\frac{310 \times 10}{23} = \frac{3100}{23} = 134.8 \text{ mEq/L}$$

Milliequivalents and millimoles. Results for the determination of sodium (Na+), potassium (K+), choloride (Cl−), and HCO_3- are often expressed in equivalents per liter. The preferred Scientific Internationale (SI)/International Unit (IU) unit is moles per liter. The univalent ions in the milliequivalents per liter and the millimoles per liter are numerically the same. Thus, milliequivalents per liter equals millimoles. The divalent ions, for example calcium (Ca++), magnesium (Mg++), and inorganic phosphorous (HPO_4=) were usually reported in milligrams per deciliter (mg/dl). Phosphate, however, reported as milligrams P per deciliter. If it is preferred that milliequivalents per liter be used for reporting calcium, phosphorous, or magnesium, then the formula below may be used:

$$\text{mEq/L} = \frac{(\text{mg/dl}) \times 10}{\text{Atomatic weight}/\surd}$$

The symbol $\surd$ is the valence. For example, the preferred unit for these quantities is now also millimoles per liter, and thus these divalent ions (for example, Ca++ and Mg++). Thus:

$$\text{M mole/L} = \frac{(\text{mg/dl})\ \text{X}\ 10}{\text{Atomic weight}}$$

For example, a value of 12 mg/dl of calcium is equal to the given quantity in the calculation below in mEq/L and M mole/L. Calcium has an atomic weight of 40 and a valence of 2.

$$\text{mEq/L} = \frac{12\ \text{X}\ 10}{40/2} = \frac{120}{20} = 6.0\text{mEq/L}$$

$$\text{M mol/L} = \frac{12\ \text{X}\ 10}{40} = \frac{120}{40} = 3.0\ \text{M mole/L}$$

Exercises (051):

1. How many milliequivalents per liter are equal to 8 mg/dl of calcium?

2. How many millimoles per liter are equal to 8 mg/dl of calcium?

3. How many millimoles per liter are equal to 4.0 mg/dl of inorganic phosphorus?

4. If the normal values for inorganic phosphorus in adults are 2.5 to 4.8 mg/dl, what are the normal values in M mol/L?

5-6. Hydrogen and Hydroxyl Ion Concentration

You have had some experience with the hydrogen ion concentration and buffer systems. The routine urinalysis always includes a pH determination. As you know, standard acids and bases are indispensable reagents in any chemistry laboratory. Also, because of the essential relationship of pH and buffer systems to the study of enzymes, you must understand these factors to appreciate an enzyme system. Urinalysis, standard acids, and buffers are not unfamiliar terms to you. It remains, then, for you to become more familiar with some of the implications of these terms. This section will define pH, standard acids and bases, and buffer systems and discuss some of the relative considerations.

052. Identify terms related to hydrogen and hydroxyl ion concentration and buffer systems.

pH. The term "pH" is taken from an expression which literally means "presence of hydrogen." This, in effect, is a definition of pH, for it is an indication of the hydrogen ion concentration of a solution. As a means of relating changes in hydrogen concentration in small meaningful numbers, the Danish scientist, Sorensen, devised the pH scale in use today. This scale runs from 0 to 14, a pH of 14 being one in which the hydrogen ion concentration is 1 X 10^{-14} moles per liter. A pH of 7 is considered neutral, because the concentration of hydrogen ions that pure water furnishes upon dissociation is 1 X 10^{-7} moles per liter. The formula 1 X 10^{-7} is also the concentration of hydroxyl ions as a dissociation product of pure water. Since the hydrogen and hydroxyl ion concentrations are equal, the pH is neutral. Table 5-1 shows the pH scale in relation to the moles per liter ion concentration. In mathematics, an exponent to the base 10 is a logarithm. It is, therefore, also possible to write pH as a minus log of the hydrogen ion concentration, or in symbols $\text{pH} = -\log [\text{H}+]$. Remember that concentrations inclosed in brackets symbolize moles per liter. A knowledge of logarithms makes it possible to convert pH to moles per liter, or moles per liter to pH.

Indicators. Chemicals that vary in color, depending upon the hydrogen ion concentration, are known as *indicators*. Note in table 5-2 that each of the various indicators has a pH range over which it has a characteristic color. If several such indicators are incorporated in a paper strip, the result is pH paper commonly used in chemistry laboratories. Individually, indicators are useful in that if the pH at which a color change occurs is known, an indicator can be selected to signal that point. For example, diethylaminoazobenzene (Toper's reagent) is red below a pH of 2.8 and a salmon pink at a pH of 2.8 to 3.0. Above

TABLE 5-1
pH SCALE RELATED TO ION CONCENTRATION

pH	H^+ Ion Concentration	OH^- Ion Concentration
0	1×10^{-0}	1×10^{-14}
1	1×10^{-1}	1×10^{-13}
2	1×10^{-2}	1×10^{-12}
3	1×10^{-3}	1×10^{-11}
4	1×10^{-4}	1×10^{-10}
5	1×10^{-5}	1×10^{-9}
6	1×10^{-6}	1×10^{-8}
7	1×10^{-7}	1×10^{-7}
8	1×10^{-8}	1×10^{-6}
9	1×10^{-9}	1×10^{-5}
10	1×10^{-10}	1×10^{-4}
11	1×10^{-11}	1×10^{-3}
12	1×10^{-12}	1×10^{-2}
13	1×10^{-13}	1×10^{-1}
14	1×10^{-14}	1×10^{-0}

this, it becomes yellow. Hence, Topfer's reagent is used as an indicator in gastric analysis. The application of electrometric measurement is discussed in another chapter.

Buffers. A buffer is a solution that does not change pH upon the addition of significant quantities of hydrogen or hydroxyl ions. We frequently say buffers resist a change in pH. But how, exactly, is a buffer able to do this? The answer depends upon the very nature of a buffer. It is composed of a weak acid or a weak base and the salt of that acid or base. An example of a buffer is acetic acid and sodium acetate. If a strong acid is added to the mixture, some of the acetate ions will combine with the hydrogen ions to form acetic acid, a weaker acid. If a base is added to this buffer, some of the acetic acid will shift to furnish acetate ions and water. Graphically, the following represents a buffer system.

$$Na+ + Ac- + HAc \longleftrightarrow Na+ + H+ + 2Ac-$$

The addition of hydroxyl ions or hydrogen ions results in equations (a) and (b) respectively.

$$\text{(a)}\ (OH)- + HAc \longleftrightarrow Ac- + H_2O$$

$$\text{(b)}\ H+ + Ac- \longleftrightarrow HAc$$

A buffer is most efficient when the concentration of the acid is equal to the concentration of the salt. This point is referred to as the pKa value of a particular buffer and is a characteristic of the buffer. In other words, at this point the addition of hydrogen or hydroxyl ions would produce the smallest change in pH, as shown in figure 5-1. As you can see in this figure, the midpoint of equal molar concentrations of a weak acid and salt is indicated as 1.0. It is impossible to overemphasize the significance of buffers. At least a basic knowledge of what buffers are and how they function is essential both in the laboratory and in the study of physiology. Many, if not all, biological reactions will not occur if the pH is uncontrolled by a buffer system. Buffers also maintain the stability of reagents on the laboratory shelf. Some indicators may affect the pH of an unbuffered dilute solution. In such cases, electrometric methods are preferred over indicators. If a pH meter is unavailable and the solution is not buffered, it is desirable to adjust the pH of the solution being measured to minimize the effect of the indicator on the unbuffered solution.

Exercises (052):

1. Match statements in column A concerning hydrogen and hydroxyl ion concentration and buffer systems with the appropriate terms in column B.

Column A

______(1) Means "presence of hydrogen."
______(2) Sorenson scale.
______(3) Chemical which varies in color according to hydrogen ion concentration.
______(4) An indicator useful in gastric analysis.
______(5) Ratio of acid to salt at which a buffer is most efficient; pKa value.
______(6) pH of 7 and is also the concentration of hydroxyl ions as a dissociation product of pure water.
______(7) Solution that resists a change in pH.
______(8) Essential for many biological reactions and to maintain stability of reagents on shelf.
______(9) May affect the pH of an unbuffered solution.
______(10) Preferred over indicator when determining pH of buffered solution.
______(11) When the hydrogen and hydroxyl concentrations are equal.

Column B

a. Buffer.
b. Topfer's reagent.
c. Functions of buffer.
d. 1:1.
e. pH scale of 0 to 14.
f. Some indicators.
g. 1×10^{-7} moles/L.
h. pH.
i. pH neutral.
j. Electrometric methods.

TABLE 5-2
DATA ON INDICATOR SOLUTIONS

Indicator	pH Range	Acid to Base Color Change	How to Prepare
Bromcresol green	3. 8 - 5. 4	Yellow to green	0. 1 Gm., 2. 9 ml. N/20 NaOH, q. s. to 250 ml. with dist. H_2O
Bromcresol purple	5. 2 - 6. 8	Yellow to purple	0. 1 Gm., 3. 7 ml. N/20 NaOH, q. s. to 250 ml. with dist. H_2O
Bromphenol blue	3. 0 - 4. 6	Yellow to blue	0. 1 Gm., 3 ml. N/20 NaOH, q. s. to 250 ml. with dist. H_2O
Bromthymol blue	6. 0 - 7. 6	Yellow to blue	0. 04 Gm. in 100 ml. of absolute alcohol
Congo red	3. 4 - 4. 5	Blue to red	0. 5 Gm., 90 ml. water, 10 ml. 95% alcohol
Chlorophenol red	5. 0 - 6. 6	Yellow to red	0. 1 Gm., 4. 8 ml. N/20 NaOH, q. s. to 250 ml. with dist. H_2O
Cresol red	7. 2 - 8. 8	Yellow to red	0. 1 Gm., 5. 3 ml. N/20 NaOH, q. s. to 250 ml. with dist. H_2O
Litmus	4. 5 - 8. 3	Red to blue	
Methyl orange	3. 0 - 4. 4	Red to yellow	0. 1 Gm. in 100 ml. water
Methyl red	4. 4 - 6. 2	Red to yellow	0. 05 Gm. in 100 ml. 50% alcohol
Neutral red	6. 8 - 8. 0	Red to yellow	0. 5 Gm. in 300 ml. 95% alcohol
Phenol red	6. 8 - 8. 4	Yellow to red	0. 1 Gm., 5. 7 ml. N/20 NaOH, q. s. to 250 ml. with dist. H_2O
Phenolphthalein	8. 3 - 10. 0	Colorless to red	0. 1 Gm. in 100 ml. 50% alcohol
Thymol blue	1. 2 - 2. 8	Red to yellow	0. 1 Gm., 4. 3 ml. N/20 NaOH, q. s. to 250 ml. with dist. H_2O
Thymol blue	8. 0 - 9. 6	Yellow to blue	0. 1 Gm. 4. 3 ml. N/20 NaOH, q. s. to 250 ml. with dist. H_2O
Topfer's reagent	2. 9 - 4. 2	Red to yellow	0. 5 Gm. in 100 ml. 95% alcohol
Thymolphthalein	9. 3 - 10. 5	Colorless to blue	0. 1 Gm. in 100 ml. absolute alcohol

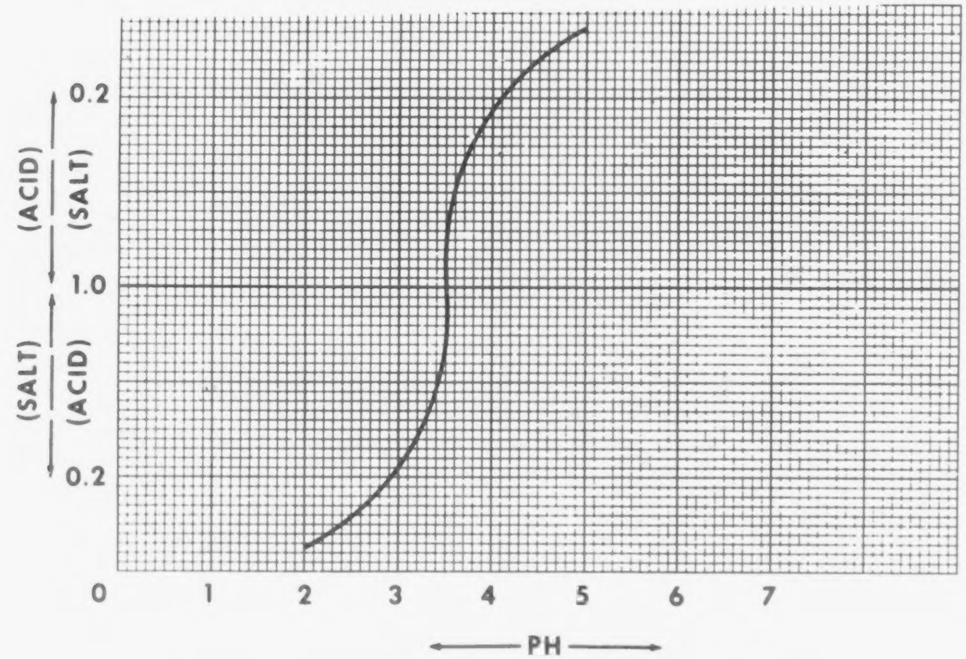


Figure 5–1. Approximate efficiency curve for a buffer of pKA = 3.5 pH units.

053. Calculate the normality of a given acid or base using the standard titration formula.

Standardizing Acids and Bases. Obviously, you need to have a known standard from which to calculate unknowns. Assuming that you are careful and accurate in your work, only one standard (called a primary reference standard), either acid or base, is necessary to determine the exact normality of all other acids and bases. A sufficient quantity of 1.0N acid or base can be accurately diluted to 0.1N and 0.01N to satisfy most requirements in the clinical laboratory.

Preparation. Standard acid or base may be secured from a reliable commercial source, though some laboratories prefer to prepare their own. One solution commonly prepared as a standard is 1N oxalic acid. Oxalic acid is available as a pure crystalline substance which can be conveniently weighed and dissolved in pure distilled water. It is advisable to use triple distilled deionized water, which is the kind used for injection purposes, if there is any doubt about the neutrality or purity of the distilled water available in the hospital laboratory. Compounds often used as acid primary standards may be seen in table 5-3.

All chemical compounds cannot be assumed to be pure enough to use in preparing a standard. Sodium hydroxide, for example, contains significant amounts of impurities, including sodium carbonate. The exact normality of sodium hydroxide is determined by titration rather than by calculation of weight. For information regarding approximate normality of acid and base reagents, see table 5-4. In many procedures, particularly bicarbonate titration, the normality must be exact. It is well for a technician to be able to distinguish situations in which the normality of a reagent is critical from a situation where the acid or base used need not be exact. In this way, valuable time will not be wasted attempting to achieve unnecessary accuracy.

All standardized acids and bases should be labeled with the date prepared and should be checked as often as necessary. Whenever a sediment appears in the bottle or contamination is suspected, the reagent must be evaluated, and it is quite likely that it must be replaced. A common source of contamination results from the use of contaminated pipette. Other common sources of contamination are created when stoppers are switched and when stoppers are placed on dirty surfaces. The philosophy that reagents are acceptable as long as controls are within range is not sound. We shall explain this more fully in Chapter 8 dealing with the quality control.

Titrations. The process of neutralizing an acid with a base, or a base with an acid, for the purpose of determining the concentration of one of them, based on a known value for the other, is an example of a titration. This is not necessarily a complete definition, because some titrations do not involve acids and bases. For example, in the chloride procedure of Schales, sodium chloride is titrated with mercuric nitrate. However, the principles involved are essentially the same for all titrations. An indicator is used to signal the point at which neutralization has taken place. This is termed the end point. It is reasonable that if it requires 5 ml of an acid of known concentration in terms of equivalent weight to neutralize 5 ml of an unknown base,

TABLE 5-3
SOME OF THE ACIDS USED AS PRIMARY STANDARDS

Primary Standard for Bases	Molecular Weight*	Gram-Equivalent Weight
Potassium acid phthalate $C_6H_4(COOH)COOK$	204.22	204.22
Oxalic acid $(COOH)_2 \cdot 2H_2O$	126.07	63.035
Potassium bitartrate $(COOH \cdot CHOH \cdot CHOH \cdot COOK)$	188.176	188.176

*Always refer to label on reagent bottle.

TABLE 5-4
APPROXIMATE NORMALITY OF CONCENTRATED ACIDS AND BASES

Concentrated Acid or Base	Approx. Normality	Ml. Required to Prepare 1 L. of a 1 N. Solution
Acetic acid	17.4	58
Ammonium hydroxide	14.8	68
Hydrochloric acid	12.1	83
Lactic acid	11.4	88
Nitric acid	15.7	64
Perchloric acid	11.6	87
Phosphoric acid	44.0	23
Potassium hydroxide (saturated at 25°C.)	13.5	75
Sodium hydroxide (saturated at 25°C.)	19.1	53
Sulfuric acid	36.0	28

the concentrations are equal. This is true because of the *law of combining weights*, which states that one equivalent weight combines with one equivalent weight. The formula applied is as follows:

Normality of acid × volume of acid = normality of base × volume of base at the end point.

Example:

10 ml of an acid will neutralize 20 ml of an 0.1N base. What is the normality of the acid?

Answer:

$$10x = (20)(0.1)$$
$$x = 0.2 \text{ normal acid}$$

Phenolphthalein is commonly used as the indicator. The base is most often titrated into the acid with a burette or pipette rather than the acid into the base. This is done because phenolphthalein is colorless in acid solution, and it is considered easier to titrate to a color than to the disappearance of a color. Actually, the titration can be performed either way. Keep in mind that concentration must be expressed in terms of normality in the titration formula rather than in molarity. Do not confuse this formula with the V–C formula used in preparing solutions of lesser concentration from solutions of greater concentration.

Exercises (053):

1. If 40 ml of an acid neutralizes 80 ml of 0.5N base, what is the normality of the acid?
2. What is the normality of NaOH if 10 ml of it neutralizes 5 ml of 1N H_2SO_4?
3. If 5 ml of 10N HNO_3 added to 10 ml of water neutralizes 25 ml of base, what is the normality of the base?
4. When titrating an unknown base against 0.1N HCl, it is found that three successive titrations require 6.30, 6.35, and 6.25 ml of the base to neutralize three 5-ml portions of the acid. What is the average normality of the base?
5. Twenty milliliters of an acid is neutralized with 30 ml of 0.5N base. What is the normality of the acid?
6. If 5 ml of 0.1M H_2SO_4 is neutralized with 15 ml of base, what is the normality of the acid?
7. What is the normality of NaOH if 10 ml of it neutralizes 5 ml of a 1M H_2SO_4?

Photometry and Spectrophotometry

A SIGNIFICANT NUMBER of quantitative determinations involve the measurement of radiant energy (light) emitted, transmitted, absorbed, or reflected under controlled conditions. Before the actual instrumentation, you must understand light as a form of energy. A better knowledge of its nature, origin, and behavior is helpful to further understand the manner in which it is used in the techniques mentioned with the use of various laboratory instruments. This chapter begins with a discussion of the concept of photometry, and later explains the basic principles of spectrophotometry and other photometric measurement techniques.

6-1. Photometry

Photometry is simply the measurement of light intensity; the simplest photometer would be a light meter such as that which you may have seen used in photography. In the laboratory, the relative light intensity is usually measured after passage of the light through a solution. A brief review of some of the characteristics of light will make this section more understandable.

054. Cite the basic characteristics of light in terms of its properties, electromagnetic energy, wavelengths, and components of visible light.

Basic Characteristics of Light. Light is a form of electromagnetic energy that travels in waves. The wavelength, or distance between the peaks of a light wave, is a function of the energy of that light. Like radio "waves," the waveform of light has properties of wavelength, frequency, and energy. These properties are all interrelated (frequency and energy are a function of wavelength) and determine the actual "character" of electromagnetic energy. For example, the actual wavelength of visible light is what determines the perceived color of the light seen by the human eye, just as the wavelength determines the nature of the electromagnetic energy.

The light from the sun is normally considered to be pure white light because sunlight contains all wavelengths. When white light, such as that from the sun, hits a prism, a rainbow, or *spectrum*, of colors is produced. As will be shown later, this is because the different wavelengths are diffracted (or bent) to different extents, and each color is seen separately. Every shade of color represents a wavelength, and the spectrum actually represents a span of constantly decreasing wavelengths, which may be called a continuum of energy.

Wavelength. Figure 6-1 shows the characteristic waveform of visible light. As illustrated in this figure, wavelength is the distance from the peak of one wave to the peak of the next. In the biological and medical sciences, electromagnetic energy in or near the visible light spectrum is most commonly expressed in nanometers (1 nm equals 1 × 10^{-3}m). (The "m" in this formula stands for micron.)

Visible light. Visible light or light that is detectable by the human eye occupies the portion of the electromagnetic spectrum from 723 nm to 397 nm. This range of wavelengths covers the range from red to violet and, exclusive of ultraviolet (UV) or infrared (IR) spectrophotometry, is the range of wavelengths used for all spectrophotometric measurements. Ultraviolet and infrared energies, although not visible, for all practical purposes may be called light. The UV range of wavelengths lies below 397 nm and is therefore invisible to the human eye, while IR wavelengths lie above 723 nm and are also invisible. Within the visible light spectrum between 397 and 723 nm, as the wavelength varies, the eye distinguishes different colors as shown in figure 6-2. Table 6-1 shows the relationship of the wavelengths to names assigned to certain areas in the electromagnetic radiation spectrum.

Exercises (054):

1. What are properties of the waveform of light?

2. What two properties are a function of wavelength?

3. What property determines the nature of the electromagnetic energy?

4. What determines the perceived color of the light seen by the human eye?

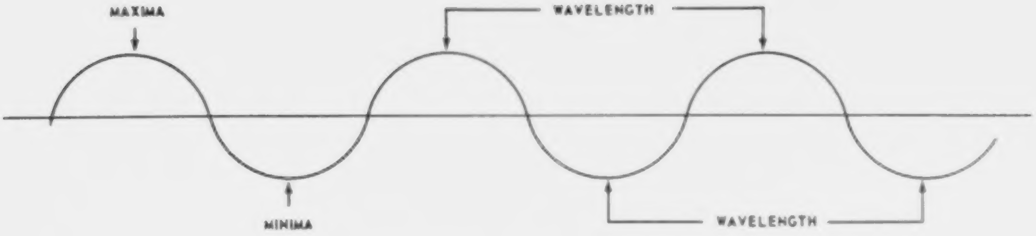


Figure 6–1. Diagrammatic representation of light showing principles of wavelength measurement.

5. When white light from the sun hits a prism, what is produced?

6. How is electromagnetic energy in or near the visible light spectrum most commonly expressed?

7. What is the range of visible light detectable by the human eye?

8. The UV range of wavelengths lies below what approximate wavelength?

055. Compare colorimeters and spectrophotometers and state the principle of the light-transmitting property of a solution and the effect of the number of molecules or ions of absorbing substances as related to the absorption of light and to concentration.

Basis for Photometric and Spectrophotometric Measurements. Instruments that use filters for isolation of part of the spectrum are referred to as photometers or colorimeters, whereas instruments that use gratings and prisms are called spectrophotometers. In relation to analytical chemistry, photometry refers to the measurements of the light-transmitting power of a solution

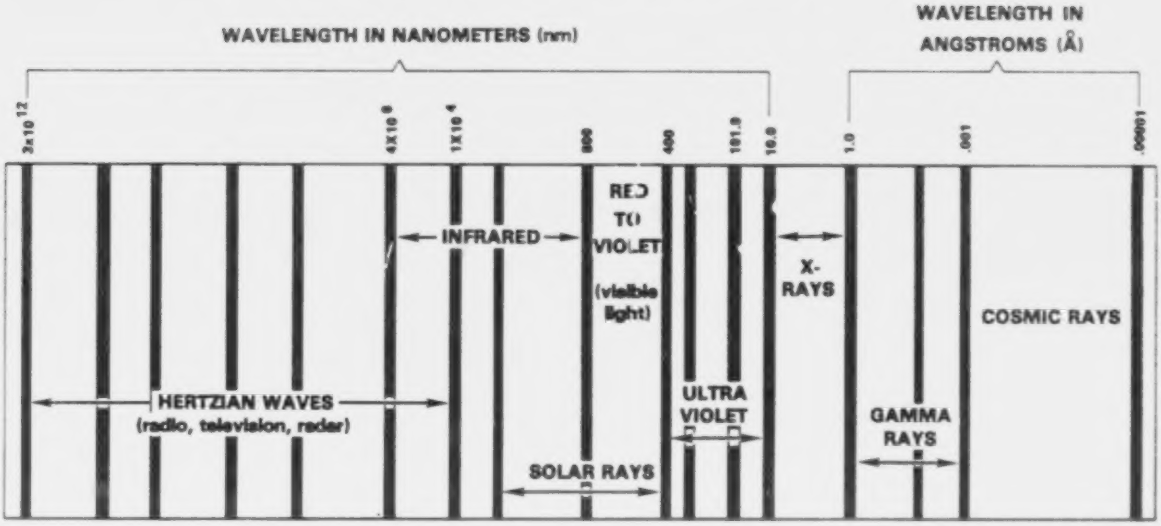
COLOR	WAVELENGTH
INFRARED (SHORT)	750-2000 nm (INVISIBLE)
RED	750-620 nm
ORANGE	620-600 nm
YELLOW	600-580 nm
GREEN	580-500 nm
BLUE	500-440 nm
VIOLET	440-400 nm
ULTRA VIOLET (LONG UV—ULTRA SHORT UV)	400-180 nm (INVISIBLE)

NOTE - Wavelength ranges are only approximate

Figure 6–2. Light waves and wave lengths.

TABLE 6-1

ELECTROMAGNETIC RADIATION CHARACTERISTICS


WAVELENGTH IN NANOMETERS (nm)
WAVELENGTH IN ANGSTROMS (Å)
$3x10^{12}$
$4X10^8$
$1X10^4$
800
400
101.0
10.0
1.0
.001
.00001
RED TO VIOLET (visible light)
INFRARED
X-RAYS
COSMIC RAYS
HERTZIAN WAVES (radio, television, radar)
ULTRA VIOLET
GAMMA RAYS
SOLAR RAYS

in order to determine the concentration of light-absorbing substances present within. Molecules in a solution will absorb light energy. The same kind of molecule will absorb the same amount of light. Thus, if one knows how much light a 1-percent solution will absorb, one can predict that a 2-percent solution will absorb twice as much.

Absorbance as measured in photometers involves not only the absorbance of the solute in the solution being evaluated but also the molecules of the liquid through which the light passes. It is, therefore necessary to adjust the instrument by means of a "blank." The greater the number of molecules or ions of absorbing substance present, the greater is the absorption of light. In other words, the deeper the color the greater is the deflection of the galvanometer from its original setting. Thus, the concentration of the absorbing component present in a solution can be measured accurately by a photometer, providing the necessary monochromatic light is used. Thus, we can see that these principles relate to Beer's law, which combined with Lambert's law states that the optical density of a colored solution is directly proportional to the number of colored molecules or ions in the path of light.

Exercises (055):

1. What is the difference between colorimeters and spectrophotometers?

2. How is photometry explained relative to analytical chemistry?

3. How do molecules in a solution react to light energy?

4. The greater the number of molecules or ions of absorbing substances present in a solution, the greater is the absorption of what component?

5. To what structure is the optical density proportional when Beer's law combines with Lambert's law?

056. Cite the purpose of colorimeter, the essential parts of a colorimeter and the principal advantages of spectrophotometric measurements.

Basic Elements of Colorimeters. When a colorimeter is referred to in the clinical laboratory, it is generally meant to be an instrument for measuring light absorption in a liquid. It is further implied that colored glass filters are used to produce the incident light of the color desired. Colorimeters all have essentially six parts. They are the power supply, exciter lamp, monochromator, sample holder, photodetector, and readout. These components may be very elementary or sophisticated. The term *colorimeter* may be used in a generic sense to include instruments of all types that measure light in the manner as described.

Spectrophotometric Measurements. Spectrophotometric measurements have gained significantly in popularity in recent years. The principal advantages of spectrophotometric measurements are relatively high sensitivity, ease with which rapid measurement can be made, and relatively high degree of specificity. Nearly all substances of interest will either absorb energy of a specific wavelength themselves or can be chemically converted to compounds that will then absorb energy of a specific wavelength. Since only a few wavelengths have been absorbed significantly by the sample, the fractional change in light intensity at the detector is much less than if only the highly absorbed wavelengths are being monitored. Thus, for maximum specificity, the spectrophotometer must produce light of limited wavelength for interaction with the sample. This light makes it easier for Beer's law to be functional. We shall discuss mathematical applications and deviations from Beer's law in greater detail later in this section.

Exercises (056):

1. What specific reaction in a liquid solution is a colorimeter generally used to measure?

2. What are the six essential parts of a colorimeter?

3. What are three principal advantages of spectrophotometric measurements?

4. What substances of interest do not directly react themselves in the spectrophotometric measurement, they are converted to compounds which react in what specific manner?

057. Identify the specific components that are common to all spectrophotometers in terms of their purpose, additional parts, and the advantages and disadvantages of certain components.

Components of Spectrophotometers. There are certain components that are common to all spectrophotometers, as shown in figure 6-3. The light, provided usually by a lamp, is separated (resolved) into its component wavelengths by the wavelength selector known as a monochromator. A small group of adjacent wavelengths, known as a band of light, then is directed at the solution in a transparent container called a cuvette. The solution absorbs part of the light. The detector measures the light intensity since the electrical signal output of the detector is dependent upon the intensity of the light which strikes or is incident upon it. The electrical signal is read as an absorbance and is proportional to the concentration of the absorbing substance in the cuvette. The light is guided, trimmed, and focused through the major components of all spectrophotometers through the use of common devices such as mirrors, slits, and lenses.

Light source. A tungsten-filament lamp is used for the visible region while a hydrogen or deuterium-charged lamp is commonly used for the ultraviolet region. Every time the wavelength changes, the zero absorbance (or 100 %T) must be set because the intensity of the light incident on the cuvette changes with the wavelength. This does not apply to the double-beam spectrophotometer.

Wavelength selector. The wavelength selector serves to isolate specific wavelengths or wavelength bands of light from the source.

A filter or a monochromator is the principal component. Transmission or interference-type filters are used, and monochromators are composed of either prisms or gratings. The filters are mirror or glass devices, each of which passes only a certain band of wavelengths of light. Transmission filters are colored glass or gelatin sandwiched between plates of glass. Light outside the transmission band is absorbed by the colored material in the filter and thereby removed. Interference filters are composed of two half-silvered pieces of glass with a dielectric material sandwiched between. Only certain wavelength bands of light emerge, depending on the thickness of the dielectric process.

Prisms and diffraction gratings both function by dispersing the light into its component wavelengths. Much narrower wavelength bands are produced when compared with filters. Another advantage is that a given prism or diffraction grating can provide a whole spectrum of wavelengths, any of which can be isolated and directed at the cuvette. Glass-prism monochromators are used for the visible spectrum, but quartz prisms are required for the ultraviolet region. Complex optical and mechanical devices are required with a prism monochromator.

A diffraction grating consists either of a transparent or a reflecting plate whose surface is ruled with a large number of closely and equally spaced fine parallel lines, each separated by a fine edge, like teeth on a saw. The edge of each of these lines reflects the incident light in all directions so that each edge functions as a source of light. Despite the fact that diffraction gratings produce a spectrum that is not curved and crowded in the longer wavelength region, they tend to produce more stray light then prisms do.

A specific wavelength band is isolated from the spectrum of a prism or grating monochromator by directing the spectrum to a plate that contains a narrow slit. Thus, only the desired wavelength is allowed to enter the sample beam, eliminating the stray light.

Cuvette. The transparent sample container is called a cuvette. It is made of glass or transparent plastic and quartz

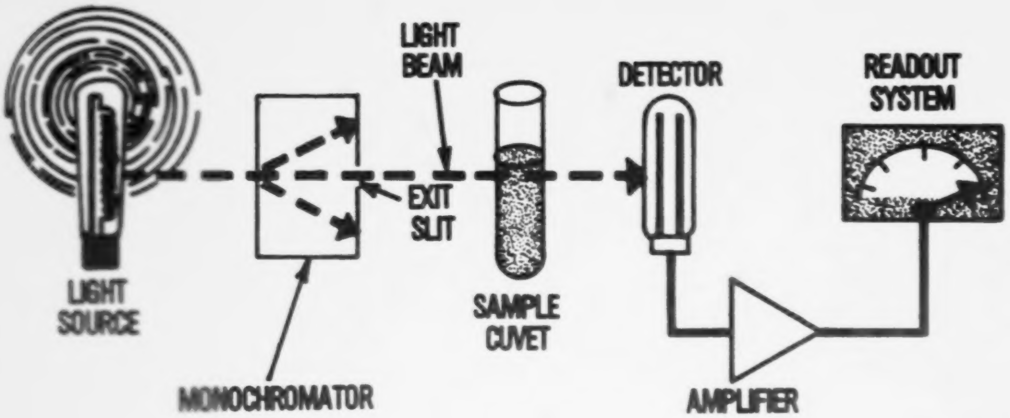


Figure 6–3. Components of a single-beam spectrophotometer.

and is either square or round. The plastic or glass measurements range from 320 to 1000 nm and the quartz measurements are below 320 nm. Square cuvettes are recommended for more accurate work since they present flat surfaces to the light. When light encounters a flat rather than a round surface, there is less tendency for the light to be disturbed by reflection, refraction, or lens effects. The operator can line up the same side of the square cuvette toward the light much easier than with the round cuvette.

Detector. The detector measures light intensity by converting the light signal into an electrical signal. As the light intensity increases, the stronger the electrical signal becomes. The two most common devices used are barrier-layer cells and photomultiplier tubes.

Readout device. The percent transmission or absorbance may be read out from the detector as a result of the electrical signal. The readout itself may be a digital display, a needle, a meter or galvanometer scale, or an ink signal on the chart paper of a recorder.

Exercises (057):

1. Match each component part of a spectrophotometer or related item in column B with the statements in column A. Each element in column B may be used once, more than once, or not at all.

Column A

_____ (1) Major components of all spectrophotometers through which light is guided, trimmed, and focused.
_____ (2) A tungsten-filament lamp is used for the visible region while a hydrogen or deuterium-charged lamp is commonly used for the ultraviolet region.
_____ (3) Serves to isolate specific wavelengths or wavelength bands of light from the source.
_____ (4) A filter or a monochromator is the principal component of this unit.
_____ (5) Are composed of either prisms or gratings.
_____ (6) Are mirror or glass devices, each of which passes only a certain band of wavelengths of light.
_____ (7) Are colored glass or gelatin sandwiched between plates of glass.
_____ (8) Are composed of two half-silvered pieces of glass with a dielectric material sandwiched between.

Column B

a. Cuvette.
b. Detector.
c. Light source.
d. Wavelength selector.
e. Monochromators.
f. Filters.
g. Prisms.
h. Diffraction grating.
i. Readout device.
j. Ultraviolet light.
k. Mirrors, slits, and lenses.
l. Lamps, cathodes, and lenses.
m. Transmission filters.
n. Interference filters.

Column A

_____ (9) Function by dispersing the light into its component wavelengths.

_____ (10) When compared with filters, they produce much narrower wavelength bands.

_____ (11) Can provide a whole spectrum of wavelengths, any of which can be isolated and directed at the cuvette.

_____ (12) Consists either of a transparent or reflecting plate whose surface is ruled with a large number of closely and equally spaced fine parallel lines.

_____ (13) They tend to produce more stray light.

_____ (14) The transparent sample container.

_____ (15) Square ones are recommended for more accurate work, since they present flat surfaces to the light.

_____ (16) Measures light intensity by converting the light signal into an electrical signal.

_____ (17) Two most common types used are barrier-layer cells and photomultiplier tubes.

_____ (18) This unit may be a digital display, a needle, a meter or galvanometer scale, or an ink signal on the chart paper of a recorder.

6-2. Calculators and Curves

Spectrophotometric calculations are derived from using Beer's law. You will master the simple mathematical equation required to calculate concentrations from photometric readings. Another way of calculating results is to run a series of standards and plot the concentration of these standards against the absorbance on linear cross-section paper or against percent transmission on semilog paper and draw a smooth curve through the points. The important feature of this section is for you to understand these spectrophotometric principles as they relate to both nonautomated and automated techniques.

058. Cite limitations of Beer's law, the technique to follow when reaction mixtures exceed the linear range in concentration, and solve problems in connection with spectrophotometric calculations to determine the concentrations of the standard, the unknown, the V factor, and dilutions.

Spectrophotometer Calculations. The absorbance (A) is directly proportional to the concentration, if the color development conforms to Beer's law. In the case of two solutions, prepared by the same procedure and read at the same wavelength, the relationship between their absorbances, A_1 and A_2 and their concentrations, C_1 and C_2 is as follows:

$$\frac{A_1}{A_2} = \frac{C_1}{C_2}$$

This may be expressed as:

$$\frac{OD_u}{OD_s} \times C_s = C_u$$

where

OD_u = optical density of the unknown
OD_s = optical density of the standard
C_s = concentration of the standard
C_u = concentration of the unknown

The answer will always be in the same terms as concentration of the standard. For example, if a standard is expressed in mg/dl, the value of the unknown will be mg/dl. There are certain limitations to this whole concept, however. Beer's law is not valid at extremely high concentrations. How do you know at which concentration deviation will take place? This must be determined for each procedure when the procedure is established. Reaction mixtures exceeding the linear range in concentration may rarely be diluted if you expect accuracy. In most cases, it is necessary to repeat the entire procedure using a dilution of the original specimen; this procedure is recommended.

Another limitation to Beer's law is that it applies only to clear solutions. Turbidity changes the linear relationship between color and concentration because light rays are scattered by suspended particles (Tyndall effect). Concentrations of turbid solutions are measured with special devices, or they may be estimated with a spectrophotometer. But, strictly speaking, Beer's law is not followed for turbid solutions. If turbidity happens to develop along with a color, the test is performed again, or the solution must be cleared by filtration or centrifugation. Returning to Beer's law, and assuming that it applies to the particular test, you may express C_s in milligrams (or other units) of standard present. As is the case with any other statement of concentration, C_2 is an expression of the amount of solute present. C_2 equals the amount of standard in 1 ml times the number of milliliters used in the procedure. As stated earlier, C_2 may be given in mg/dl, and is arrived at as follows:

$$\text{Ml of std used} \times \text{mg per ml in std} \times 100 = \text{mg/dl}$$

You would encounter a complication only if, in actually performing the test, you used a volume of unknown different from the volume of standard. You would then need to correct for the difference in volume between the unknown and the standard with a V factor. Use Appendix G, sections 13 and 14, *Summary of General Formulae*, for additional information. Mathematically, this factor is as follows:

$$V = \frac{\text{ml serum or actual specimen} \times \text{diluted specimen used}}{\text{total volume of diluted specimen}}$$

For example, if 2 ml of serum are used in preparing 20 ml of a protein-free filtrate (PFF), and 1 ml of the PFF is used, the V factor is:

$$V = \frac{(2)\quad(1)}{20} = 0.1$$

This V factor is placed in the original spectrophotometer formula as follows:

$$C_u = \frac{OD_u}{OD_s} \times C_s \times \frac{100}{V}$$

Remember, the V factor is necessary to take into consideration any difference in volume between the amount of the standard and the amount of unknown specimen used in the procedure.

One final factor enters into the spectrophotometer formula. It is quite simple and seldom applies. If the final volume of the unknown solution differs from the final volume of the standard, a dilution factor is supplied. The formula then becomes:

$$C_u = \frac{OD_u}{OD_s} \times C_s \times \frac{100}{V} \times \frac{\text{final volume of unknown}}{\text{final volume of standard}}$$

Usually, the unknown is treated exactly like the standard, and there is no need to correct for different final volumes. But if the unknown should be diluted with an equal volume, for example, multiplication of C_u by 2 would correct the equation. (See Appendix H, Mathematics, for brief review of mathematical equations related to the spectrophotometer formula.)

Exercises (058):

1. In the spectrophotometer calculations when color development conforms to Beer's law, the absorbance is directly proportional to what specific component?
2. How do extremely high concentrations affect Beer's law?
3. What general technique is recommended when reaction mixtures exceed the linear in concentration?
4. Why does turbidity in a solution change the linear relationship between color and concentration?
5. How is the turbidity removed if it develops along with color?
6. What does C_s (Concentration of the standard) equal?
7. What is the formula for the V factor?
8. In a BUN procedure, the standard consists of 0.05 mg nitrogen per milliliter, and 3 ml are used in the test. Calculate the C_s factor.
9. If 1 ml of serum is used in a test to make a 1:10 PFF, and 2 ml of the PFF are used, what is the V factor?

059. Specify the significance of a calibration curve, the type of paper used of plotting percent transmittance curves, the relationship between concentration and percent transmittance as compared with absorbance and concentration, and the purpose of a spectral-absorbance curve.

Calibration Curves. A calibration curve relates to A (absorbance) or %T (percent transmittance) and is necessary in quantitative work where the amount of absorption has to be calculated. If the dilution factor, depth of cuvette, and chemical aspects of a procedure (for example, incubation time) are kept constant, the standard solution should theoretically read the same from day to day. In reality, standards are seldom that reliable for all procedures. But for purposes of introducing the concept of calibration curves, we shall assume that the standard of a particular concentration consistently yields a particular optical density reading. A second standard of one-half the concentration should be read at one-half the optical density; a third standard of one-fourth the concentration of the first should be read at one-fourth the optical density. As a result, if a series of such standards is charted on graph paper, the results will be a straight line for any test procedure which follows Beer's law, as shown in figure 6-4. Specimens treated in the same way as the standard could be read from this same curve. All curves used in the clinical laboratory are not necessarily straight line segments. Many photometers and spectrophotometers have scales reading directly in absorbance values on units proportional to absorbance. Percent T can be plotted versus concentration on semilogarithmic graph paper as shown in figure 6-5. One cycle semilog paper is sufficient if %T values all fall in the range 10–100.

In most instances it is unsatisfactory to plot %T versus concentration as shown by a nonlinear curve in figure 6-6. Six or more points are required to draw an accurate curve, and it is difficult to give proper experimental weight to each point. In addition, it is virtually impossible from such a plot to decide whether Beer's law applies, and results must be calculated by graphical reference to the curve itself. In the event there is a significant change in one or more of the standards used to check such a curve, it is necessary to reconstruct the entire curve in order to determine its proper shape. For these reasons a plot of absorbance versus concentration is much preferable.

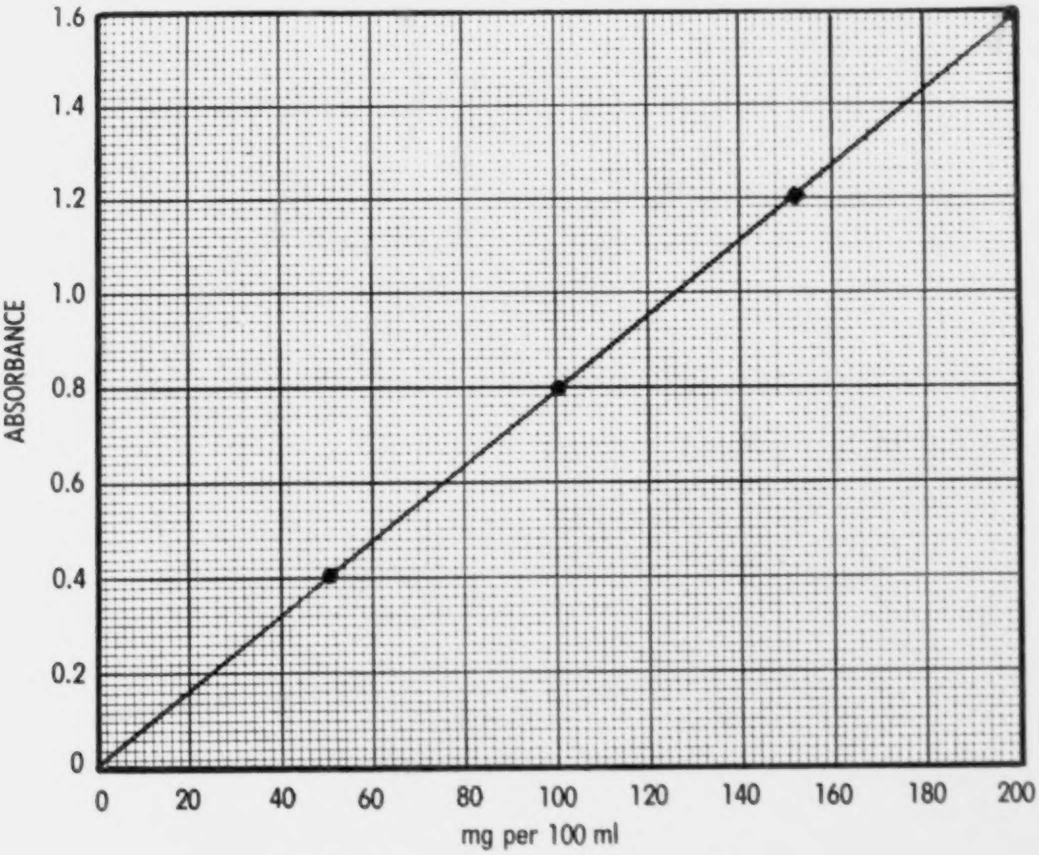


Figure 6–4. Calibration curve, absorbance versus concentration.

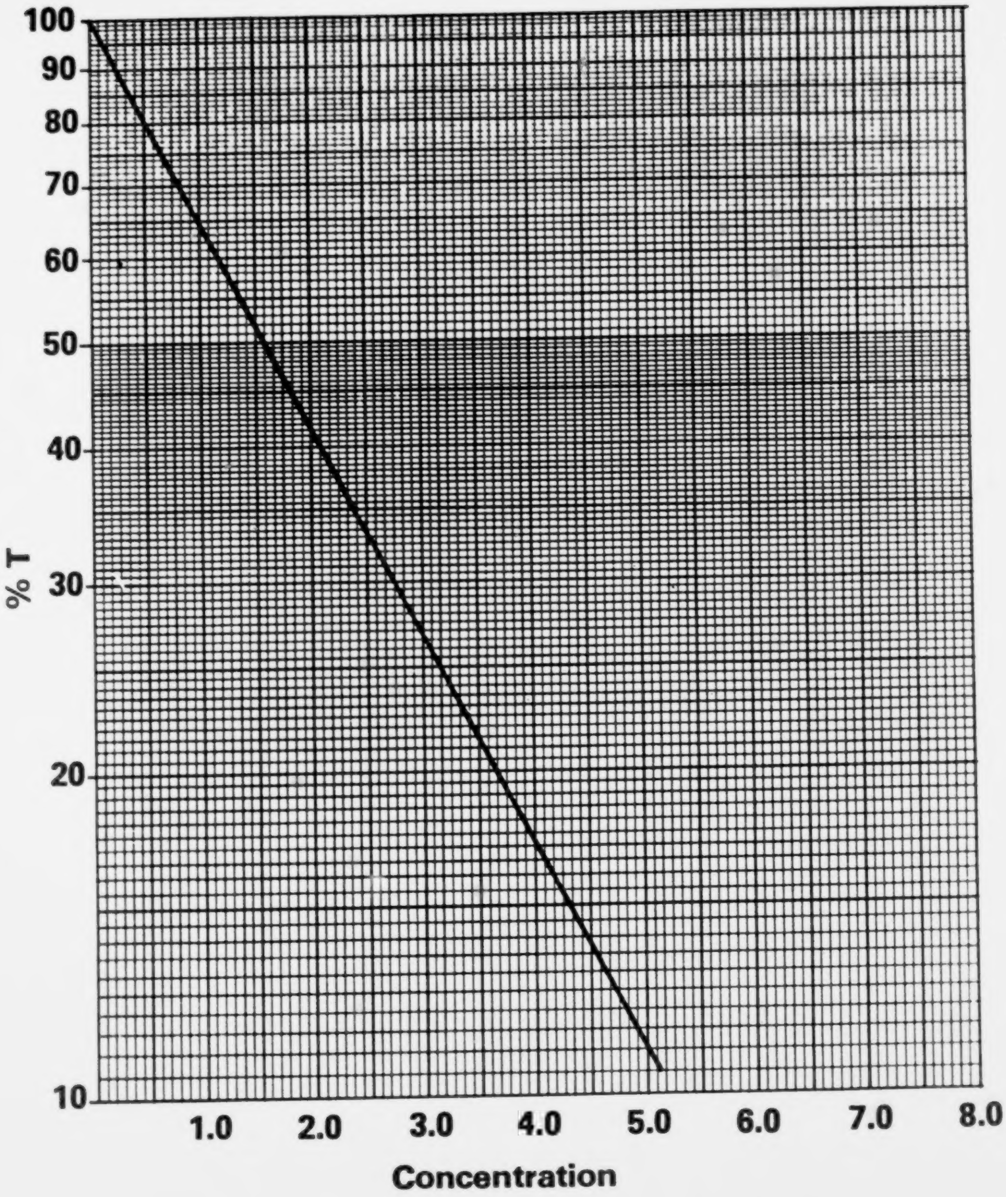


Figure 6–5. Calibration curve, percent transmittance versus concentration (linear).

A curve in which concentration is plotted versus absorption is called a concentration-absorbance (C–A) curve. The straight line (linear) curve mentioned in the previous paragraph and illustrated in figure 6-4 is a C–A curve. It is used in lieu of calculations involving the spectrophotometer formula, but it should be used with caution, because what holds true under one set of circumstances may not hold true under another. In the case of a curve that does not follow Beer's law, as shown in figure 6-5, the mathematics involved is too complicated to achieve a reportable result effectively without the aid of a graph. Thus, we have at least two reasons for using C–A curves. They save time, and they simplify calculations. Used properly, C–A curves are of great value to the clinical laboratory. The spectrophotometer scale is usually calibrated in percent T (%T) as well as optical density. It is generally easier to read percent transmittance, but as stated previously, the relationship between concentration and percent transmittance is not a direct proportion as it is between concentration and optical density. This is because optical density equals the expression log T or log 1/T. In this respect several factors should be noted.

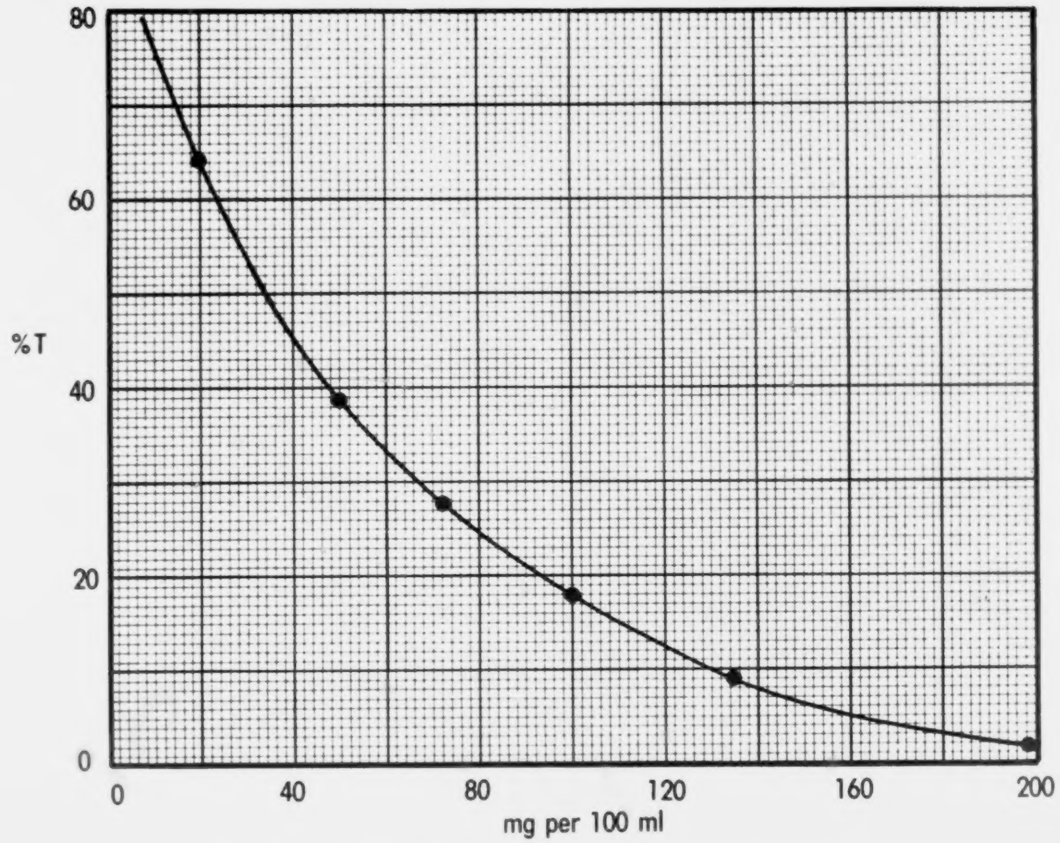


Figure 6–6. Calibration curve, percent transmittance versus concentration (nonlinear).

a. Optical density is synonymous with absorbancy. In the literature of this field, various designations are assigned to this value, such as OD, D, A_s a_s a, A, or E (extinction).

b. Optical density (or absorption) is logarithmically proportional to transmittance or percent transmittance.

c. T in this formula refers to transmittance and *not* to percent transmittance (%T). Percent transmittance is 100 × T (percent T = 100 × T). This quantity may be referred to as T, T_s, or I. Thus, it is possible to achieve a linear graph if percent T is plotted on semilog graph paper rather than on ordinary graph paper (C–T curve). Such a graph is just as useful as any other type except that semilog graph paper is harder to read. It is important to observe that labeling of the verticle or y-axis begins at 10. The midpoint between the lower left-hand corner and 20 percent T is not 10, as can be seen by a study of figure 6-5. Errors are frequently made in labeling the y-axis.

Spectral-Absorbance (S–A) and Spectral-Transmittance (S–T) Curves. A spectral-absorbance curve enables you to obtain the wavelength at which maximum absorption takes place with a given solution. You have just learned that maximum benefit is derived from Beer's law if a wavelength of maximum absorption is used. In a sense, this is a trial-and-error sort of curve in which the optical density (OD) is recorded as the wavelength and is changed in small increments. The wavelength at which maximum optical density is observed is then chosen as the wavelength for that particular determination, providing no further correction for interference because of natural or extraneous color is required. If percent transmittance is plotted versus wavelength, instead of OD versus wavelength, an inverted curve results, but all of the principles are the same. Such a curve is called a spectral-transmittance curve. It is not necessary to plot the S–T curve on semilog paper because no linear relationship is shown. The various points are plotted as shown in figure 6-7. The purpose of the plot is limited to showing the lowest point or points of the curve. this is particularly important if absorption is in the infrared or ultraviolet range, although S–T curves are valuable at various wavelengths.

Exercises (059):

1. To what two spectrophotometric principles does a calibration curve relate and for what purpose?

2. On what type of paper is percent T versus concentration plotted?

3. What should be done if there is a significant change in one or more standards used to check a calibration curve?

4. What are two given reasons for using C–A curves?

5. How is optical density related to absorbancy?

6. How is optical density related to transmittance?

7. Which one is direct, when the relationship between concentration and percent transmittance and the relationship between concentration and optical density are compared?

8. What purpose does a spectral-absorbance curve serve?

9. What happens if percent transmittance is plotted versus wavelength instead of OD?

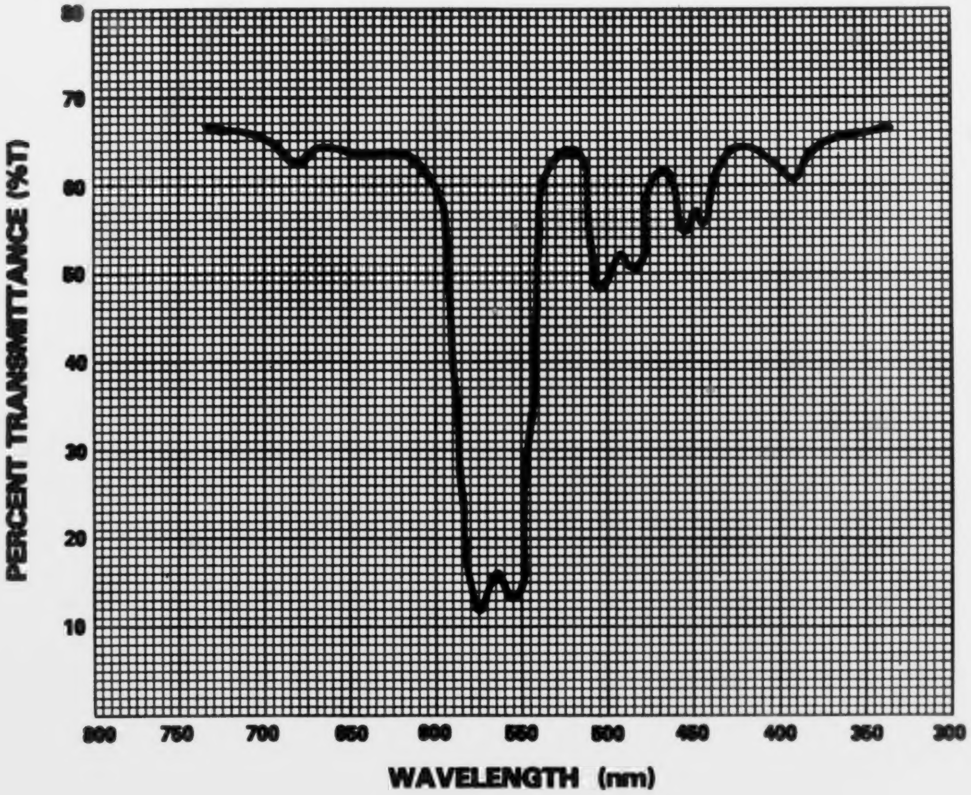


Figure 6–7. Spectral-absorbance curve.

060. Explain the significance of the steps to follow for the care and proper operation of a spectrophotometer in terms of the wavelength calibration, linearity, bandwidth, and stray light checks.

Spectrophotometer Care and Operation. In order to insure accurate and reliable operation, several checks should be made from time to time in the normal operation of a spectrophotometer.

Wavelength calibration. If the instrument is in routine, daily use, some type of wavelength check should be performed each day. Use a didymium filter or some such standard to check absorbance wavelengths. This is considered sufficient for a routine calibration check. These values should be recorded. Any significant changes should be investigated. Most quality-built instruments will go for long periods without the need for recalibration; nevertheless, the routine checks must be done. Whenever lamps are changed, or instruments repaired or moved about, the wavelength calibration must be checked.

Linearity. Whenever the standard curve is prepared, one is checking the linearity of the instrument. If the substance being used follows Beer's law, the absorbance plotted against concentration should be a straight line. It is very important that you observe deviations carefully to see if there is a tendency for repeated plots to show the same deviation from linearity, which might indicate a lack of linearity of the instrument. You should always remember that readings below 20 %T and over 60 %T are inherently somewhat in error, the error being significant at the end of the scale. Accurately prepared standards should be purchased and used periodically as a careful linearity check. All results should be recorded and kept permanently.

Band width. The band width is defined as the peak width at 50 %T. In order to check this characteristic properly, a standard source lamp (which may be purchased for use) or a sharp cut-off filter should be available. If a mercury lamp is used, a good isolated peak should be found at 546 nm. Turn the 100 %T adjustment knob to set the meter on 100. Then move the wavelength setting toward 700 nm until the meter reads 50 %T. Record the wavelength. Now move the wavelength setting toward 400 nm until the meter again reads 50 %T. Record this wavelength. The difference between the two recorded wavelengths is the band width. It should correspond fairly closely to the band width claimed by the manufacturer. The spectrum in most instruments is significantly distorted, so the band width at the red end of the spectrum may be noticeable narrower than at the violet end.

Stray light. Reflection within the instrument, light from the next higher-order spectrum, or room light reaching the detectors all may cause stray light. Blocking filters are used to absorb light at any given wavelength. A procedure as prescribed by the instrument manufacturer using blocking filters should be made periodically to insure proper function.

Exercises (060):

1. If a spectrophotometer is in routine, daily use, how often should the wavelength be checked?

2. Under what other specified circumstances should the wavelength calibration be checked?

3. When a standard curve is prepared, what specific check is made on the spectrophotometer?

4. What type of reagents should be used for the linearity check?

5. What must be done with the results obtained from the linearity check?

6. What is the band width?

7. What %T readings are considered to be inherently somewhat in error?

8. After the band width check is done, to what reading should the result correspond?

9. What condition causes stray light?

10. What device is used to absorb light at any given wavelength?

061. Differentiate from among the given commonsense checks the most appropriate action to take in order to insure accurate and reliable operation of a spectrophotometer.

Commonsense Checks. Even though you, as a laboratory technician, will be busy from time to time, it is important that you make the time-consuming checks to insure that your instrument functions properly. There are very simple, commonsense observations that can be made that show serious problems. Making these commonsense checks will insure the successful operation of your instrument as a fisherman would instinctively check his boat for leaks before going all the way out from the shoreline. Let's consider the following checks.

Warmup time. Turn your instrument on and set the meter at 100 %T. Check for several minutes to see how long it requires to stabilize. Do not use it until the meter stays at zero.

Fatigue. Does the instrument have a tendency to fade below the initial setting if it is left on for some time with the meter on 100 %T? The instrument may be usable if fading is very slow, but 100 %T should be reset often during use.

Meter repeatability. Place a sample in the cuvette well and set the meter at 50 %T. Remove the sample and reinsert it. Check the meter. If the needle does not return to 50 %T, the instrument should be serviced by the biomedical equipment repair technician.

Grating deterioration. Insert a slip of white paper into the cuvette well so you can observe the color of light entering the well. Rotate the wavelength knob slowly from 400 to 750 nm. You should learn to recognize the approximate colors of various wavelengths. A grass-green color should be noted at 555 nm. A yellow-orange should be noted at 610 nm. Colores should be sharp and clear. If more than one color appears and colors are streaky, the grating may be deteriorating or dusty. The instrument should be checked by a biomedical equipment repair technician.

Low energy. Insert an empty cuvette in the well and set the wavelength at 400 nm. Turn the 100 %T set knob fully clockwise. The needle should go past 100 %T with ease well before the knob is fully clockwise. Repeat at 700 nm. If the needle reaches 100 %T only when the knob is fully rotated clockwise, there is insufficient energy. The exciter lamp may be dirty or going bad, the slit may be dusty, the amplifier (if there is one) may be weak, or the detector may be defective. Efforts should be made to isolate the trouble.

Scratches or defective cuvettes. Insert a water blank in the well and set the meter at 50 %T. Rotate the cuvette slowly and watch the needle. If it moves more than 1 %T, it should be discarded. Some cuvettes have an index line, and they should be oriented in the well in the same way each time. All lab techs should be alerted to this as a possible source of error.

Loose connections. Set the meter about midscale, thump on the table close to the instrument enough to jar the instrument slightly. Move the power cord and watch for meter response. If the needle moves at all and does not return to its exact position, the instrument should be serviced.

Defective controls. Does the instrument respond to the positions indicated when operating the on and off switch? When setting the zero or 100 %T knobs, does the meter respond smoothly and quickly? If the needle moves erratically, the controls should be checked for possible replacement.

As with any other precision instrument in the chemistry laboratory, a spectrophotometer should be kept clean and dust free. Do not allow solutions to drip or leak into the cuvette well. The instrument should be used on a solid counter or bench in a location where it will not be bumped or jostled. It is good practice to have the instrument cleaned and the dust brushed or blown from out of its interior by the biomedical equipment repair technician.

Exercises (061):

1. Match each commonsense spectrophotometer check in column B with the statements in column A. Each element in column B may be used once, more than once, or not at all.

Column A

_____ (1) Check for several minutes to see how long it takes for the instrument to stabilize.

_____ (2) Does the instrument have a tendency to fade below the initial setting if it is left on for some time?

_____ (3) The initial step requires placing a sample in the cuvette well and setting the meter at 50 %T.

_____ (4) The type of check you would be making when you observe the color of light entering the cuvette well.

_____ (5) This condition could be possible when more than one color appears and colors are streaky.

_____ (6) This check is demonstrated when the instrument needle is observed to reach 100 %T only when the knob is fully rotated.

_____ (7) This check requires that the cuvette be rotated slowly and the needle be observed for movement greater than 1 %T.

_____ (8) The power cord is moved and the immediate response is observed. The needle moves and does not return to its exact position. This completes what check?

_____ (9) Which check should be made if the needle moves erratically?

Column B

a. Warmup time.
b. Defective controls.
c. Fatigue.
d. Loose connection.
e. Meter repeatability.
f. Scratched or defective cuvette.
g. Grating deterioration.
h. Low energy.
i. High energy.
j. Scratched exciter lamp.

Column A

_____ (10) A technician making this check sets the meter about midscale, and thumps on the table close to the instrument enough to jar the instrument slightly.

062. Cite steps in the preliminary operation of the Gilford Stasar III spectrophotometer in terms of given specifications, guidelines, and purpose.

Spectrophotometer Stasar III. The Gilford Stasar spectrophotometer (Stasar III) has gained good acceptance in many Air Force clinical laboratories. It is among one of the first popular spectrophotometers designed primarily for microsamples. Diligently follow the manufacturer's guidelines for preventive maintenance and the operating procedures, and you can expect long and reliable service. Poor maintenance and operator error are primary causes of failures and malfunctions. The Stasar III spectrophotometer is shown in figure 6-8. A 500-microliter (μl) sample allows a 1 cm light path. The spectral range is from 335 nm to 700 nm with a band pass of 8 nm. Linear measurements of absorbance or concentration are displayed on a bright, easy to read light emitting diode (LED) readout. Samples are drawn through a measuring cell by a vacuum pump or appropriately available vacuum outside the instrument.

This instrument has been coupled with samplers, microprocessors, and printers to produce small automated chemistry analyzer systems. Let's briefly review the preliminary operation of the instrument.

Preliminary operation. Before operating the instrument you should review the operator's manual to become familiar with basic operation principles and instruction notes.

a. Turn the instrument on and allow it to warm up for 15 minutes.

b. Turn the vacuum pump switch on if there is one available; however, countertop vacuum can be used.

c. Adjust the vacuum and sample time to aspirate a volume which yields the best reproducibility, using a vacuum regulator as shown in figure 6-9.

Measuring absorbance. Absorbance should be measured after completing preliminary operations as follows:

a. Place the CON–ABS–ACC control shown in figure 6–10 to ABS.

b. Select the desired wavelength, using the wavelength control.

c. Purge with air for 5 seconds or more as shown in figure 6-11,A.

d. Aspirate a reference blank as shown in figure 6-11,B: insert the tip of the sample inlet tubing well below the surface of the liquid without touching the bottom of the container. Depress the actuator bar to its first stop.

e. Adjust the zero control shown in figure 6-10 for reading of 0.000 on the LED readout.

f. Purge with air for 5 seconds or more.

g. Aspirate the sample and read the absorbance. Make sure that the sample inlet tubing is well below the surface of the sample material without touching the bottom of the sample container. Erratic readings may result from insufficient sample intake.

h. Purge with air for 5 seconds or more.

i. Repeat steps *g* and *h* for continued samples. Make sure you introduce the reference blank and zero the instrument after changing wavelengths or reagents.

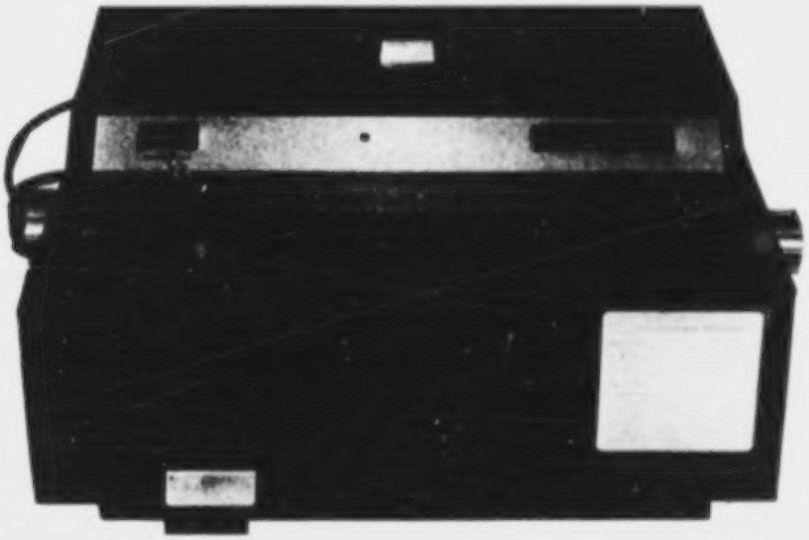


Figure 6-8. Spectrophotometer — Stasar III (Gilford Instrument Laboratories, Inc.).

End-of-Run-Cleaning. The following procedures are used for the end-of-run cleaning.

a. Dilute flo-kleen ® 1:5 as described on the label.
b. Purge 15 ml of flo-kleen ® through the cuvette.
c. Purge with air for 5 seconds.
d. Purge 25 ml of distilled water through the cuvette.

Maintenance. You must always refer to the operating directions for minor adjustment and operation of the spectrophotometer. However, all persistent malfunctions should be reported to the medical equipment repair technician with the completing of the appropriate work order. Under no circumstances should you assume the task of any major repair of the spectrophotometer. The medical equipment repair technician is trained and skilled to perform all extensive calibration maintenance and repair of such equipment.

Exercises (062):

1. What are two primary causes of equipment failures and malfunctions?

2. What is the spectral range of the Stasar III spectrophotometer?

3. How wide is the band pass?

4. What basic rule should one follow before operating the instrument?

5. How long should the instrument be allowed to warm up?

6. How should the vacuum and sample time to aspirate a volume be adjusted?

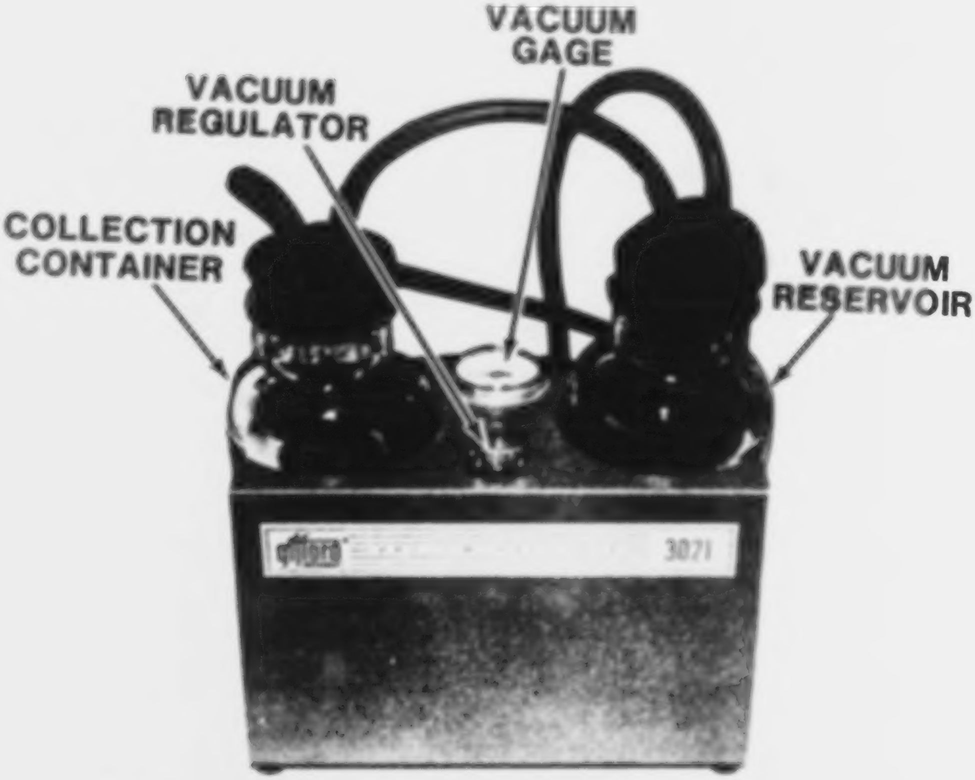


Figure 6-9. Vacuum receiver.

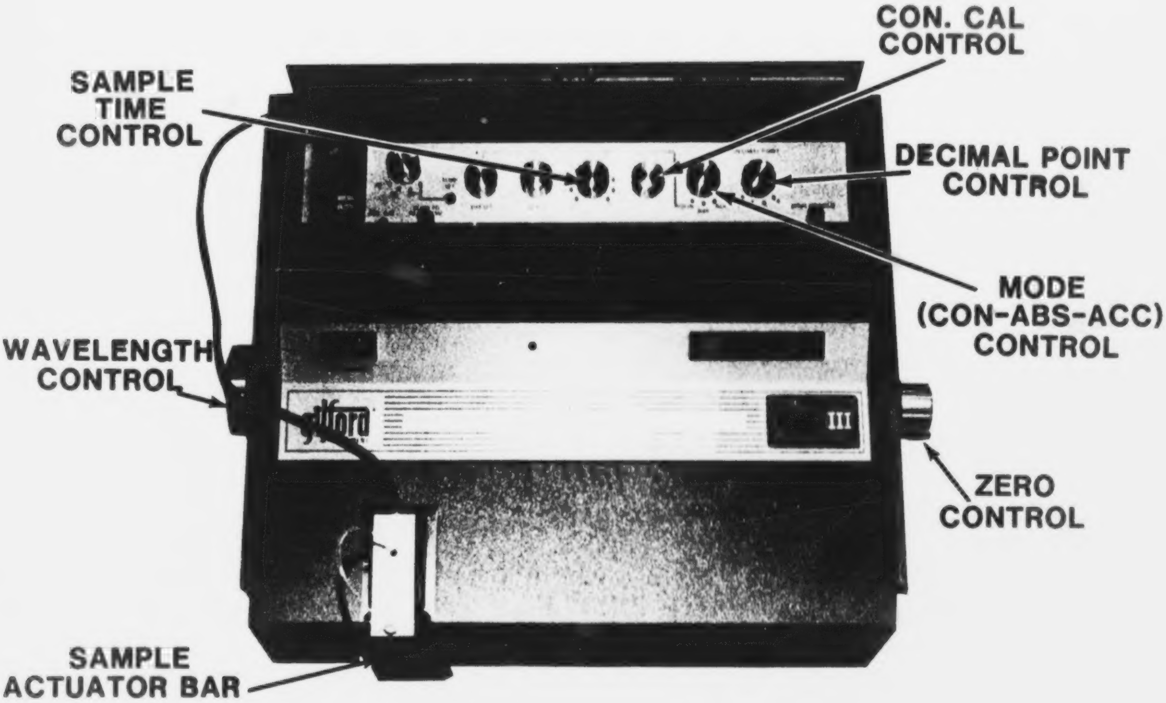


Figure 6–10. Spectrophotometer — Stasar III operating controls.

7. What is the first step in measuring absorbance after completing preliminary operations?

8. When inserting the sample inlet tubing into the sample, what precaution must be followed?

9. In the process of aspirating the sample and reading the absorbance, what condition can cause erratic readings?

10. What action should be taken after changing wavelengths and reagents?

6-3. Flame Photometry

Flame photometry is widely used in the clinical laboratory to determine sodium, potassium, and lithium concentrations in biological fluids. Thus, the flame emission spectrophotometer is one of the most important instruments used in clinical laboratories. An understanding of the principles of flame photometry will help you to further understand the mechanisms responsible for interferences.

063. State the principle of flame photometry, limitations of technic, and the functions of the components.

Principle of Flame Photometry. In flame photometry, the serum, urine, or other specimen is vaporized by aspiration into a flame to produce certain colors of light. Color that is characteristic of the light emitted by the constituent being measured is passed through a filter to a photoelectric cell where its intensity is measured. By comparing the intensity of emitted light with that of a

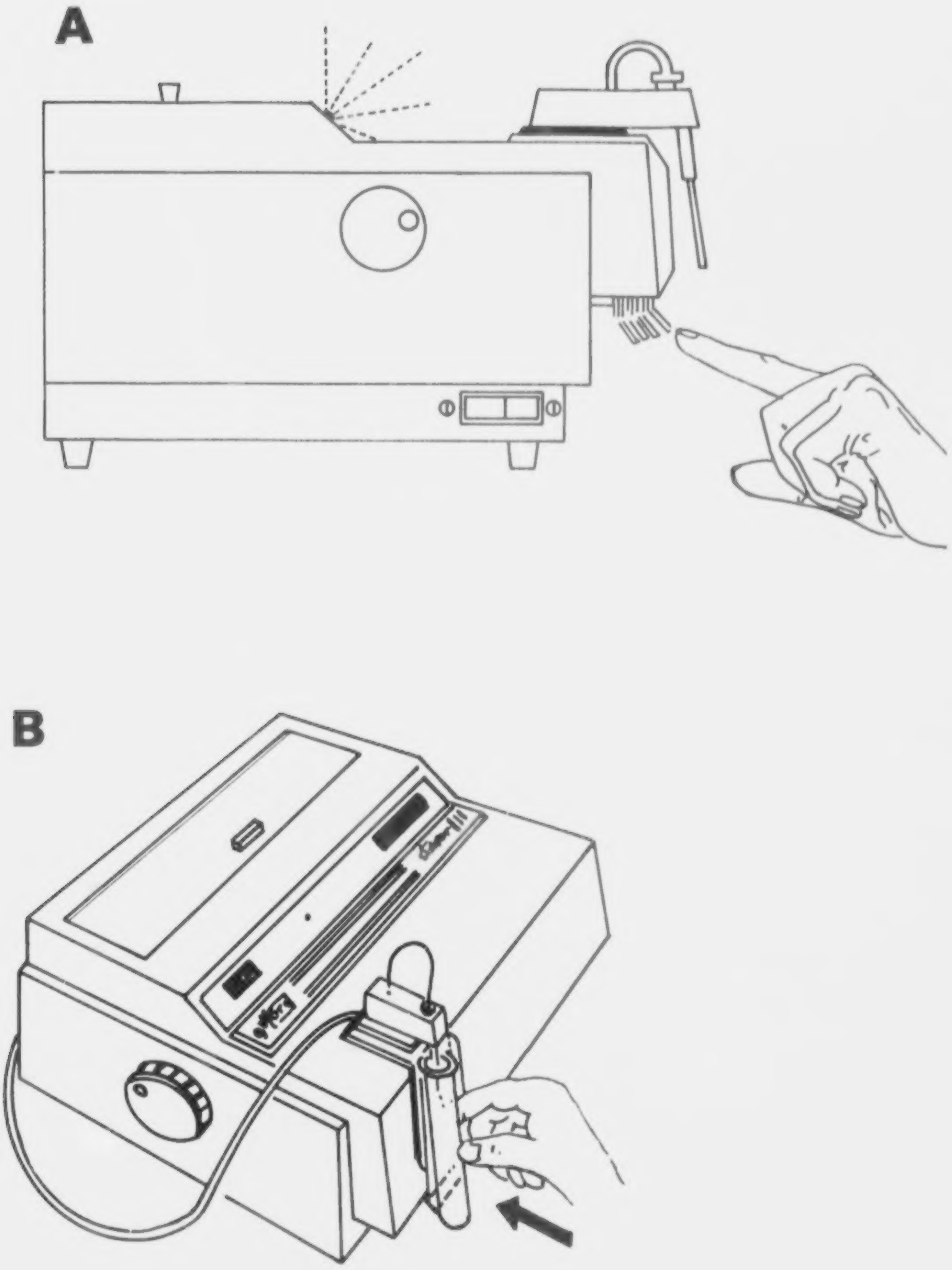


Figure 6–11. A. Purging the system.
B. Aspirating a sample.

standard, concentrations of metallic ions can be determined. When a metallic salt is burned in a flame, colors are produced. These colors are at very specific wavelengths that are characteristic of the ion being burned. The wavelength to be used for the measurement of an element, as in spectrophotometry, is dependent upon the selection of a spectral line of strong enough intensity to provide adequate sensitivity. It also depends upon freedom from other interfering lines at or near the selected wavelength. Flame photometry lends itself well to direct concentration measurement of some metals. However, cations like calcium are less easily excited in the ordinary flame. In these cases, the amount of light given off may not always provide adequate sensitivity for analysis.

The flame photometer reads directly into milliequivalents per liter, thus eliminating the need for the mathematical formula used in absorption measurements.

All modern flame photometers determine simultaneously the amounts of sodium and potassium in a sample in relation to a high concentration of a reference element. That reference element usually is lithium. The effect of sodium on potassium is reduced by the mixed sodium-potassium standard containing concentrations of elements that are similar to those found in serum. The standards are: Sodium - 140 mEq/L; potassium - 5 mEq/L. Those for urine are: 50 mEq/L each.

All flame photometers used in the clinical laboratory are of the internal standard type, in contrast to the direct type whereby the intensity of the characteristic spectral emission is used as a measure of concentration. In the internal standard type, the intensity of the spectral emission of the element to be determined is balanced against that of an added element that acts as an internal standard. The internal standard is lithium or cesium. The internal standard such as lithium or cesium acts to absorb the radiation and buffer the potassium atoms from the effects of mutual excitation.

Instrumentation. The essential components of a flame photometer are (1) the flame, (2) the burner head and atomizer, (3) the entrance slit, (4) the wavelength selector (filter), (5) the exit slit, (6) the detector, and (7) the readout device.

Natural gas, propane, and compressed air are used to produce *the flame* in most flame photometers found in the clinical laboratory. Regulators maintain a constant flow of gas, since it is essential that the flame temperature be held constant. The *atomizer* and the flame are the two most critical components in a flame photometer. The function of the atomizer is to break up the solution into fine droplets so that the atoms will absorb heat energy from the flame and become excited.

The monochromator includes the entrance and exit slits that are similar to those described for spectrophotometers. Their function is to isolate the wavelength of interest from interfering light before it is emitted on to the detector. The detector operates on the same principle as previously described in the section on spectrophotometry.

Limitations. Although there are other possibilities, the only elements commonly analyzed by this technic are sodium, potassium, and lithium. The major sources of error include (1) an unsteady flame, (2) dirty glassware, (3) clogged aspirator, and (4) hemolysis. Despite limitations, flame photometry will probably remain the method of choice for the determination of serum sodium and potassium. When properly performed, serum sodium may be measured with a coefficient of variation of 2 or 3 percent.

Exercises (063):

1. State the principle of flame photometry.

2. The wavelength to be used for the measurement of an element as in spectrophotometry is dependent upon what two factors?

3. Why are cations like calcium less easily excited in the ordinary flame?

4. What are the values of the standards used for sodium and potassium found in serum?

5. What two elements are used as internal standards?

6. What purpose does the internal standard serve?

7. Since it is essential that the flame temperature be held constant, what component maintains a constant flow of gas?

8. What are the two most critical components in the flame photometer?

9. What is the function of the atomizer?

10. What are the elements commonly analyzed by the given technic of flame photometry?

11. What are four major sources of error in the given technic of flame photometry?

064. Cite the principle of atomic absorption spectrophotometry and the elements commonly determined by this technique.

Atomic Absorption Spectrophotometry. Atomic absorption spectrophotometry is a method for determination of metallic elements in low concentration. The principles on which this method is based are closely related to those of flame emission photometry. The basic difference is that in flame photometry one measures the light emitted by the small fraction of the sample atoms in the flame that are excited to emit their characteristic radiation, whereas in atomic absorption one measures the absorption of light by the unexcited atoms. Atomic absorption may be used for the determination of calcium, magnesium, sodium, potassium, chromium, mercury, lithium, nickel, bismuth, cobalt, manganese, and some other elements in biologic material. Calcium is more accurately determined by atomic absorption than by flame photometry, since in flame photometry the presence of other elements may cause some interference with the elements being determined. Phosphate, for example, causes some interference with calcium determinations. This problem has been overcome by adding a constant amount of lanthanum to the samples and standards to combine with the calcium determinations.

Simple technics are available for the determination of elements such as lithium, magnesium, and calcium. Other elements of biologic interest such as lead, iron, and copper normally found in such low concentrations may require one or more sophisticated instruments.

Exercises (064):

1. What is the principle and purpose of atomic absorption spectrophotometry?

2. What is the basic difference between flame photometry and atomic absorption?

3. List some elements that may be measured by atomic absorption.

4. Why is calcium more accurately determined by atomic absorption than by flame photometry?

065. State the principle of flourescence; define fluorometry in terms of its function; cite the basic components of a fluorometer, the advantages of fluorometry over absorptive methods, some disadvantages of fluorometry, and uses of fluorometric technics.

Fluorometry. Fluorescence may be considered as one of the results of the interactions of light with matter. Some chemical compounds have the property of absorbing light energy and then remitting some of this energy in light of a longer wavelength than that originally absorbed. The longer wavelength results in less energy remitted. This method of analysis uses the principle of fluorescence. Fluorometry is the measurement of this fluorescence for qualitative and quantitative analytic purposes. In the process, a solution of the constituent being tested is irradiated with ultraviolet light causing it to fluoresce; the remitted visible light is passed through a suitable monochromator and measured quantitatively by photometric means. For quantitative purposes, the intensity of fluorescence is measured and compared with that emitted from solutions of known solute concentrations. For dilute solutions of limited concentrations range, the intensity of fluorescence is directly proportional to the concentration of the constituent being determined.

Usually, the exciting light is in the ultraviolet or near-ultraviolet wavelength in the range of 250–400 nm and the emitted fluorescence is of a longer wavelength, usually in the visible range. Fluorescent methods can be used to measure concentrations lower than the colorimetric methods by a factor of from 10–1000 and thus are desirable for the measurement of very low concentrations.

Instrumentation. The basic components of a fluorometer are similar to an absorption spectrophotometer. The major difference is the introduction of a set of filters or a monochromator before and after the cell to isolate the emitted light. The principal components are the exciting light, filters or monochromators to separate the exciting light from the emitted light, and a sensitive detector. The energy source of the fluorometer is generally a mercury lamp or xenon lamp that will produce enough energy that when absorption occurs, electron transitions to higher energy within the molecule will occur.

The main advantage of fluorometry over absorptive methods of measurement is increased sensitivity. The increased sensitivity is said to be as much as 10,000-fold over absorbance measurements. A second advantage of fluorometry is specificity. A substance that absorbs light but does not fluoresce will not interfere with the measurement of a fluorescent compound in a solution.

Disadvantages include interference by other fluorescent compounds, especially drugs. Many materials at low concentrations can contribute extra fluorescence or quench (diminish) the fluorescence of other substances. Turbidity and air bubbles must be avoided because they cause false scattering of light. Increasing temperature causes decreasing fluorescence. Thus, all samples must be measured at the same temperature.

Fluorometry is used to measure catecholamines, quinidine, porphyrins, procainamide, cortisol, estrogens, several other pharmaceuticals, and some of the metallic ions. A new application involves coupling fluorometry to liquid chromatography. Using this technic, a compound can be separated qualitatively with chromatography and spot measured fluorometrically.

Exercises (065):

1. Briefly explain the principle of fluorescence.
2. In the fluorometric technic, at what wavelength and range is the exciting light measured?
3. Emitted fluorescence is of a ____________ wavelength, usually in the ____________ range.
4. What is the major difference between the basic components of a fluorometer and an absorption spectrophotometer?
5. What is the energy source of the fluorometer?
6. What is the main advantage of fluorometry over absorptive methods of measurement?
7. Why should turbidity or air bubbles be avoided in the fluorescence procedure of measurement?
8. How is the test affected if the temperature is not kept constant and allowed to increase?

066. Cite the principle of ionspecific electrodes, determinations that use ion-specific electrodes, and the types of electrodes used in terms of their composition and advantages.

Ion-selective Electrodes. Ion-selective electrodes are used in the clinical laboratory for the measurement of hydrogen ion (H^+)pH, sodium (Na^+), potassium (K^+), calcium (Ca^{++}), fluoride ($F-$), and chloride ($Cl-$). Each electrode has a unique ion-selective phase that is intended to make the electrode respond to only one ion. However, interference ions do exist for all ion-selective electrodes. A significant property of these electrodes is that they are not absolutely specific.

When dipped into a solution containing a specific ion, ion-selective electrodes develop an electrical potential that is a function of the amount of that ion present. All ion-selective electrodes require a reference electrode because two electrodes are required for any measurement.

Specific applications. Flame photometry has been observed to present a number of problems, and the maintenance of flame photometers provides many operational headaches and safety hazards. A search for safer, more accurate, and less cumbersome techniques for determining sodium and potassium has led many companies to examine electrochemical methods. Since chloride and carbon dioxide determinations are almost always requested along with sodium and potassium, as an electrolyte battery, several companies have sought methods of combining all four tests into one instrument.

Sodium. The sodium electrode is currently used to measure both the activity and the concentration of Na^+ in blood and urine. The Na^+ electrode operates on a principle that is the same as that of the hydrogen ion electrode. The difference is in the contents of the glass membrane itself. Sodium, as measured by ion-specific electrodes (ISE), suffers from no interferences of any significance in normal blood-derived samples.

Potassium. Potassium is measured similarly to sodium more profitably by ISE than by flame-emission photometry. Urine potassium values greater than 50 mEq/L in ISE may not be considered to be as reliable as compared to those using flame-emission photometry. This problem can be avoided by diluting urine samples that have such high values. A liquid ion-exchange membrane electrode using the antibiotic valinomycin as the potassium binder is most selective for potassium.

Chloride. Highly selective solid-state electrodes composed of AgCl (silver chloride) have been developed to measure chloride activities in both serum and cerebrospinal fluid.

Carbon dioxide (CO_2). HCO_3- is quantitatively the second most important anionic fraction in serum. CO_2 produced by cellular metabolism diffuses into the plasma and combines with H_2O inside red blood cells to form

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H_2CO_3. The H_2CO_3 dissociates to form H^+ and HCO_2-. The CO_2 electrodes are ion-selective electrodes that are placed behind a gas-permeable membrane. A typical CO_2 electrode such as that introduced by Severinghouse consists of a pH electrode that has a flat surface, a thin layer of a weak bicarbonate buffer, and a silicone membrane covering the glass electrode surface.

The major advantage of such electrodes is that the measurement system is isolated from the sample, and even the reference; the electrode junction is inside the silicone membrane.

Exercises (066):

1. What are some tests that are measured by the technique using ion-selective electrodes?

2. What happens when ion-selective electrodes are dipped into a solution containing a specific ion?

3. How many electrodes are required for the measurement of a specific component?

4. What other two tests are almost always requested along with the sodium and potassium as an electrolyte battery?

5. What is the given difference between the sodium electrode and the hydrogen ion electrode?

6. What is the composition of the electrodes used to measure chloride activities in both serum and cerebrospinal fluid?

CHAPTER 7

Automation

DEMAND FOR laboratory data continues to grow. It is virtually impossible to perform with manual technics the large numbers of some chemical tests that are ordered daily. Hence automation is essential to the clinical chemistry laboratory. Many laboratories have had significantly large increases in the workload in recent years. Thus, an attempt to increase the number of tests that can be performed in the chemistry laboratory without great increases in personnel and space has been the prime consideration with the use of many automatic and semiautomatic devices and equipment.

Currently, the test workload has increased at a 15-percent annual rate and is doubling every 5 years. This kind of growth has been generated and sustained by improvements in productivity, which is attributed significantly to automation.

Automated analytic systems are subject to the same problems as those encountered in manual procedures in trying to maintain accuracy and precision. Factors that must be considered in the use of automated systems are sampling; delivery of reagents; removal of interfering materials; heating or incubation of reaction mixtures; and measurement, recording, and quantitation of data.

This chapter is devoted to the principle and practice of automation with particular emphasis on the types of systems and the most commonly used.

7-1. Types of Automated Systems

Automated chemical analyzers currently available utilize two distinctively different operating principles. The two types are *continuous flow* and *discrete analysis*. In the continuous flow system, samples follow each other in sequence through a channel. In discrete analysis, each sample occupies a separate container, and the containers are tested in parallel or in sequence.

067. State the function of continuous flow analysis in terms of the significant parts of the system and specify those characteristics that maintain the efficiency of the system.

Continuous Flow Analysis. Continuous flow analyzers are instruments that continuously pump reagent through tubing and coils to form a flowing stream and continuously pump samples into that stream (fig. 7-1). The essential part of a continuous flow system is a peristaltic pump composed of steel roller rods which compress several pieces of plastic tubing by rolling on them. Fluids are drawn into the tubing and pushed through the system. Each test requires a manifold of plastic tubes, one for the sample and others for the reagents. The size of the inner diameter of the tubing employed determines the amount of each sample or reagent used.

The samples are loaded into a rotating sample tray from which they are picked up in sequence by a probe attached to a manifold tube. Samples follow each other into the reagent streams, interspersed with a wash solution.

The success of the system depends entirely on small air bubbles injected into the sample and reagent streams at strategic points. These bubbles segment the stream and through a squeeze-like action on the tubing, keep the segment intact. This insures that the sample does not mix with the one following it. Simultaneously, the efficiency of the mixing of reagents in each small segment is improved. Mixing is enhanced by directing the stream through glass coils. This creates a tumbling motion in each segment, thus resulting in the mixing of the reagents and sample. The description given thus far is used in all methods for introducing samples and reagents into the system. Following this, the stream flows through various fittings and modules designed to carry out the required chemical reactions and readings.

When deproteinization is required, the stream containing the sample is directed through a semicircular groove on one side of a dialysis membrane. A stream of recipient solution flows through a similar groove on the other side of the membrane. The solute that is of interest dialyzes into the recipient stream, with the residual protein left behind.

If a period of time is required for a reaction to take place, the stream is directed through a time-delay coil. This coil is made of plastic or glass, the length and inner diameter of which determine the amount of time it will take the stream to pass through. If heating is required, a time-delay coil is immersed in a heating bath maintained at a desired temperature by a thermoregulator.

After the reaction is completed, the stream flows through an appropriate instrument for measurement. This usually is a spectrophotometer, a photofluorometer, or flame photometer. A strip chart recorder pen is activated to give visual signals, or peaks generated from the signals in these instruments. The size of these visual signals is related to the concentrations of the reactant in the samples. A series of standards accompanies each set of test to give a standard curve. The concentrations of the tests are determined from a standard curve.

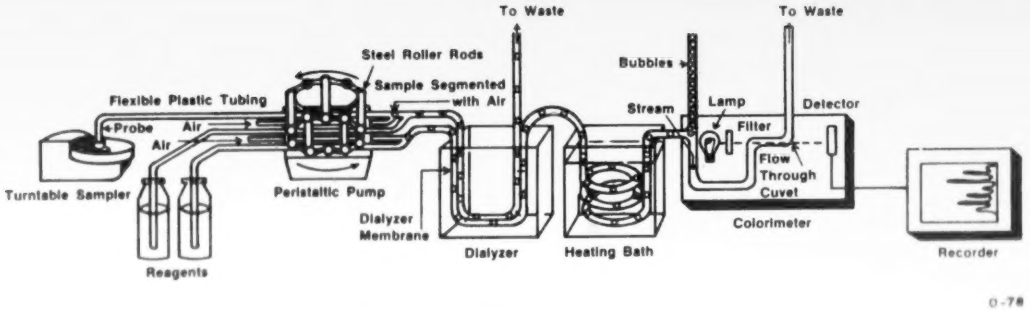


Figure 7-1. Continuous flow analysis system.

Exercises (067):

1. What are continuous flow analyzers?

2. What is the essential part of a continuous flow system?

3. What is the essential part of a continuous flow system composed of and how does it work?

4. What factor determines the amount of each sample or reagent used?

5. The success of the system depends entirely on what factor?

6. How does directing the stream through glass coils enhance mixing?

7. When deproteinization is required, a stream containing the sample is directed through a semicircular groove on one side of what type of apparatus?

8. What two factors determine the amount of time it will take for the stream to pass through the reaction time-delay coil or glass tube?

068. Compare the discrete analysis and continuous flow systems of automation and specify the sequence of steps of the sample from the initial loading into the instrument to final analysis.

Discrete Analysis. As indicated earlier, discrete analysis involves the treatment and measurement of samples in individual containers. Whereas the continuous flow system employs a proportioning system for measuring samples and reagents, discrete systems employ automatic pipetting devices. The samples are loaded into a sampler tray and pipetted by the instrument into reaction tubes. The samples may be pipetted in sequence or in parallel, depending on the type of instrument used. The reaction tubes then move through various stations where more reagents are added and other operations are carried out, such as mixing and heating.

A sample of the final solution is then removed automatically and measured using a spectrophotometer, photofluorometer, or flame photometer. The reaction vessel itself serves as a cuvettes. The readout device may be a recorder or a printout of the final results.

Note in figure 7-2 that the centrifugal fast analyzer differs from the types as described in figure 7-1. In figure 7-2, the samples and reagents are measured by an automatic pipetter into separate compartments of a teflon wheel (transfer disk). The disk is placed in the instrument where it rotates at a fixed speed. During the spinning period, the reagent flows by centrifugal force into the sample compartments. The treated samples then flow into cuvettes located in a rotor around the outside rim of the disk. The electronics of the instruments are so designed that as the cuvettes pass through the vertical light beam of a spectrophotometer, readings are made in rapid sequence and printed out as desired. This permits a single endpoint to be read a number of times or a kinetic reaction to be followed with almost continuous readings. Observe that the disk and rotor are shown in figure 7-2.

Exercises (068):

1. What is the difference between the discrete analysis and the continuous flow systems of automation?

2. In the discrete analysis, what step follows after the samples are loaded into a sampler tray?

3. What steps follow after the sample is added to the reaction tubes?

4. When the final solution is removed automatically and measured using a spectrophotometer, the reaction vessel itself serves as a ____________.

5. In the centrifugal fast analyzer where are the sample and reagents placed?

6. How does the reagent flow into the sample compartment?

7. When are sample readings done?

069. Cite the manual chemistry steps and their automated counterparts; the reason for periodic standardization of automated methods and the advantages and disadvantages of the use of automated instruments.

Considerations in the Use of Automated Instruments. You should recognize automated systems as the use of machines to accomplish the usual manual technical steps common to many chemical methods. Note that table 7-1 lists some routine requirements of chemical procedures and their automated counterparts.

Once primary conditions are set, the well-designed automatic system maintains or reproduces these conditions with immense precision. Periodic standardization of the methods is essential to insure continuing *accuracy* of results.

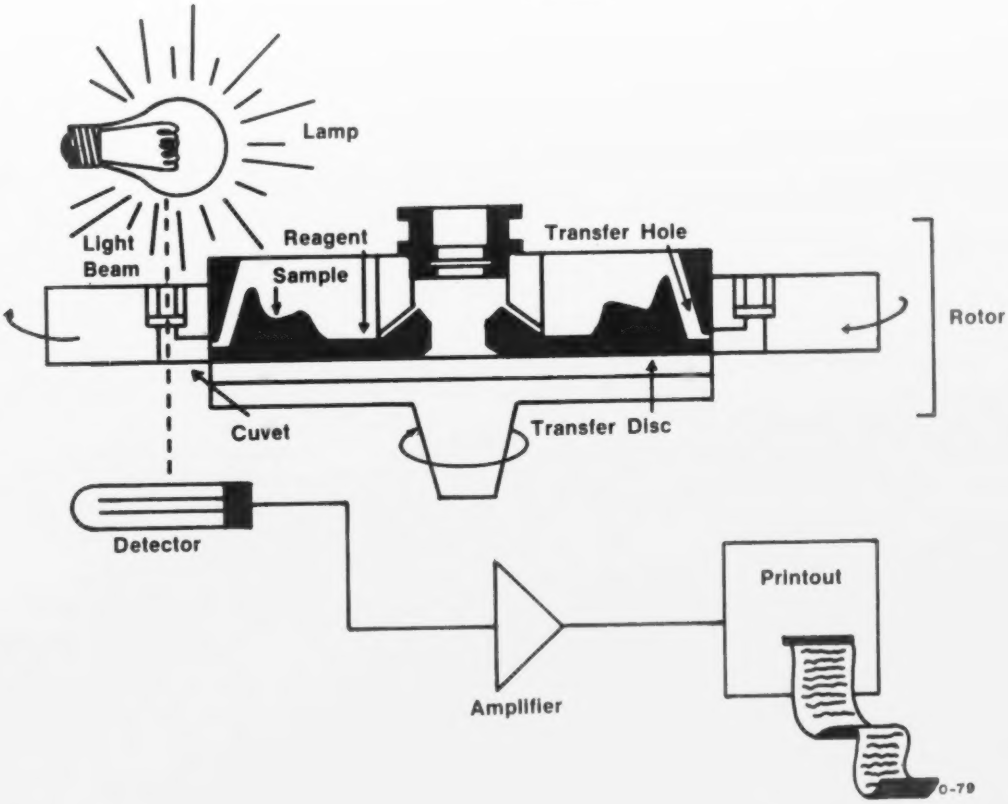


Figure 7-2. Discrete analysis system (concept of centrifugal fast analyzer).

TABLE 7-1

AUTOMATION OF MANUAL TECHNICS

MANUAL STEP	AUTOMATED
1. PIPETTING SAMPLES AND REAGENTS	1. SAMPLER, PUMP, PIPETTER
2. MIXING	2. MIXING COIL, AIR, MECHANICAL MIXER
3. DEPROTEINIZATION	3. DIALYZER, RESIN COLUMN
4. HEATING OR INCUBATION	4. HEATING BATH OR BLOCK
5. DEVELOPMENT TIME	5. DELAY COIL, CONVEYER TRAVEL
6. REACTANT MEASUREMENT	6. FLOW-THROUGH SPECTROPHOTOMETER FLUOROMETER, FLAME PHOTOMETER
7. READING AND CALCULATION	7. RECORDER, PRINTER, COMPUTER

Whether sample and reagent measurements are made with automatic pipets or by proportioning (as in the continuous flow system), the important consideration is *reproducibility*. The absolute quantities measured are not critical (within certain broad limits) as long as the measurements are precisely repeatable.

Advantages. The advantages of automation are apparent and are listed below. These advantages are to be considered valid only if the instrument or system is operating in a sound manner.

a. Large numbers of samples may be processed with minimal technician time.

b. Two or more methods may be performed simultaneously.

c. Precision is superior to that of manual performance.

d. Calculations may not be required.

Disadvantages. Laboratory personnel sometimes become so impressed with the appearance and operation of automated equipment that they fail to observe its shortcomings. Some of the problems common to many automated systems are stated below.

a. There are often limitations in the type of methodology that can be used. A compromise is sometimes made in automated chemistry procedures which results in less accurate values than with manual procedures.

b. Technicians tend to become complacent and fail to observe and exercise discretion regarding potential problems due to the automated characteristics of these instruments.

c. Due to their uniform objectivity, the instruments cannot exercise the judgement expected from an experienced technician in the case of a potentially interfering turbidity or a spurious color that can relate to a given sample or to an entire set of tests.

d. Small quantities of sample make the use of many of these systems impractical. Thus, backup manual procedures may be required for individual emergency analysis. Backup procedures must also be available in the event of instrument failures.

e. The systems are expensive to purchase and maintain. Regular maintenance schedules require technician time as well as visits from trained technical representatives of the equipment.

Automated systems produce results with relative ease and can encourage the accumulation of large amounts of irrelevant data that must be calculated, recorded, and stored.

Exercises (069):

1. By what component of the automated instrument is deproteinization accomplished?

2. Reading and calculation is done by what component of the automated instrument?

3. When development time is required, what technique of the automated instrument provides this task?

4. Why is periodic standardization of the methods essential in the operation of automated instrument?

5. What is the important consideration in either system of automation?

6. Under what conditions would the advantages of an automated instrument be considered valid?

7. Why do technicians tend to become complacent and fail to exercise discretion regarding potential problems in the operation of automated instruments?

8. What disadvantage is expressed regarding the presence of potentially interfering turbidity or spurious color in samples when automated instruments are used?

9. What should be available in the event of instrument failures?

070. Define automated analyzer terms and identify analyzers with their operational features and principles and advantages and disadvantages.

Common Automated Instruments. There are a number of automated instruments common in the clinical laboratory. Let us briefly review the terms associated with instrument categories and compare automated instruments.

Terms associated with instrument categories. When describing some automated instruments, some terms are used to describe the categories. They are dwell time, throughput-samples per hour, stat testing, and batch analyzer.

Dwell time. The dwell time is the minimum time required to obtain a result after the initial sampling of the specimen.

Throughput. Throughput is the maximum number of samples or tests that can be processed in an hour.

Stat testing. The word "stat" is an abbreviation of the latin word *statim* meaning "immediately."

Batch analyzer. This is an instrument that performs the same test simultaneously on all samples.

Technicon Auto-Analyzers and Technicon Sequential Multiple Analyzers. The Technicon Auto-Analyzers (AA) and Technician Sequential Multiple Analyzers (SMA and SMAC, that is, SMA with computer) are a group of instruments based on a continuous-flow principle.

Technicon SMA II (Technicon Instrument Corp.). The Technicon II is an updated version of the SMA 12/60. Sampling was increased to 90 per hour with a corresponding reduction in sample and reagent required. A small computer/printer is part of the total system. The dwell time is 10 minutes and the throughput is 60 samples per hour. The unit is not recommended for stat testing and the total repertoire of tests that can be performed is 23.

Technicon SMAC (Technicon Instrument Corp.). The SMAC incorporates the principles of the earlier instruments into a much improved computer-monitored and controlled system capable of accepting 150 samples per hour. The computer has self-diagnostic features that identify and localize operational problems. The dwell time is 12 minutes, and because of the considerable startup time, the SMAs discussed are not well adapted to off-hour or stat testing. The repertoire of tests is similar to that of the SMA II.

A wide range of test methods are available or have been reported for the SMA and SMAC. The most common use of the SMA and SMAC has been for profiles in the medium-size and large laboratories. For the SMAC, the throughput is 3,000 tests per hour.

Eastman Kodak Ektachem. The Eastman Kodak Ektachem is a discrete, selective analyzer that used dry-film technology. The dry reagents are rehydrated by the addition of 10 μ L of serum sample. The sample serves as a solvent. The film is composed of multiple layers, some of which serve to ultrafilter the sample (removing protein), whereas others serve to provide reactive reagents. The colored reaction's products are measured by reflectance on the side opposite the sample addition. The reflected light is converted into concentration units by the Williams-Clapper formula which is similar to Beer's law for converting light transmission into absorbance units.

Each sample is automatically processed by dispensing a drop of serum (10 μ L) from the sample cup on the slide. Slides are automatically dispensed from cartridges, each containing a different method. The slides are incubated while the color forms. Then the reflectance measurements are finally performed. Enzyme and kinetic measurements can be performed. Electrometric measurements for sodium and potassium determinations can be performed. The instrument is considered to be a discrete analyzer. Reagents are available only through the manufacturer. Mixing of reagents is accomplished through diffusion. The repertoire of tests is over 16. It has stat capability and the dwell time is 5–6 1/2 minutes. The throughput samples per hour and tests per hour is 270.

Centrifugal analyzers. There are several manufacturers of these discrete batch analyzers. The key element is a centrifuge whose rotor contains 16 to 32 cuvettes. The sample and reagent are mixed together by the action of centrifugal force when the rotor is first accelerated. A beam of light passing upward through the transparent portion of the cell measures the absorbance as the rotor is spinning. These analyzers have been especially useful for kinetic and enzyme measurements and for end-point analysis as well.

They usually perform only one kind of analysis at a time and are batch instruments. They use a small quantity of reagent, thus making them economical to operate. The dwell time is from 5 to 30 minutes. They are not well adapted for stat testing. The instrument is capable of doing over 50 tests. The throughput of samples and tests per hour is in excess of 100. The models on the market include the IL Multistat III (Instrumentation Laboratory, Lexington, Massachusetts.), the Cobas Bio (Roche Analytical Instruments, Inc., Nutley, New Jersey), the Rotochem (American Instrument Co., Silver Spring, Maryland), the Centrifi-Chem (Union Carbide Corp., New York), and the EMI Gemsaec (Electro-Nucleonics, Fairfield, New Jersey).

Du Pont ACA. The Du Pont ACA is a discrete, selective analyzer that serves as a general chemistry analyzer in small-sized hospitals and a specialty analyzer in the medium- and large-sized hospitals. Over 50 test methods are available, ranging from routine and stat chemistries to immunochemistries to therapeutic drug monitoring to coagulation. It is capable of processing 97 test or samples per hour with a dwell time of 8 minutes. Any combination of tests can be run on any sample at any time. The reagents are prepackaged in plastic packs, which serve as the reaction cuvettes. These packs contain in one or more heat-sealed compartments the reagents necessary for the specific test for which they are designed. Packs for certain tests contain individual disposable chromatographic columns, such as ion exchange or gel filtration for removal of interfering substances such as proteins. A built-in computer system reads a binary code for a particular test. The pack header contains these codes. The programmed computer directs the instrument to draw the specific volume of sample and diluent into each pack and, in succession, mixes reagents, waits a preset amount of time, forms a precise optical cell within the transparent pack walls, and measures the reaction photometrically. The computer calculates the concentration value for each test and prints out a separate report sheet for each sample, which includes patient identification data. The instrument is well capable of stat testing.

Beckman ASTRA. The ASTRA-4 and ASTRA-8 are discrete, selective analyzers that process the high-volume tests, glucose, creatinine, BUN, Na, K, Cl, and CO_2. The ASTRA-8 will process up to 72 samples per hour and up to 648 test per hour. Other samples that can be analyzed include amylase, total bilirubin, calcium, and total protein, any of which can be added to the existing ASTRA-8. The instrument is suitable for stat testing with a dwell time of 1¼ minutes.

Abbott ABA. The ABA-100 is a discrete batch analyzer that can process up to 120 tests per hour. The Abbott Laboratories systems offer the ABA-100, the ABA-200, and Abbott VP. All these use the principle of measuring the difference in absorbance at two selected wavelengths as a measure of the concentrations. This is said to eliminate much of the interference caused by turbidity and by hemoglobin or bilirubin in serum samples. This technique is called bichromatic-photometric measurement. For the ABA-100, the dwell time is from 1 to 10 minutes and is capable of stat testing. There are more than 50 tests that can be performed by the instrument.

Hitachi 705. The Hitachi 705 is a discrete, selective analyzer having a throughput of 180 tests per hour. It can have a 16-test repertoire available at any one time and, in addition, offers an ion-selective electrode accessory. Analyzers such as the Hitachi 705 have been designed for microcomputer control, and as a result the test methods can be readily changed. A significant feature of such types of analyzers is the use of a single photometer that measures multiple cuvettes at several wavelengths and multiple times. Previous analyzers used multiple photometers to process multiple tests or processed one sample at a time. A marked difference exists between this technique and that of centrifugal analyzers, which process only one test at a time.

Exercises (070):

1. What is meant by dwell time?

2. What does throughput-test per hour mean?

3. What are batch analyzers?

4. Match each of the automated analyzers in column B with the operational features, advantages, and disadvantages in column A. Some of column B items may be used more than once.

Column A	*Column B*
_____ (1) When sampling was increased to 90 per hour there was a reduction in reagent required; the dwell time is 10 minutes, but the unit is not recommended for stat testing.	a. Technicon SMA II.
_____ (2) Improved computer-monitored and controlled system capable of accepting 150 samples per hour.	b. Centrifugal analyzers.
_____ (3) The computer has self-diagnostic features that identify and localize operational problems.	c. Technicon SMAC.
_____ (4) Most common use has been for profiles in the medium-size and large laboratories.	d. Eastman Kodak Ektachem.
_____ (5) A discrete, selective analyzer that uses dry-film technology.	e. Du Pont ACA.
	f. Beckman ASTRA-8.
	g. Hitachi 705.
	h. Abbott ABA-100.

Column A

______(6) The sample serves as the solvent. The colored reactions products are measured by reflectance; instrument is a discrete analyzer; mixing is accomplished through diffusion and dwell time is 5–6 1/2 minutes.

______(7) A beam of light passing upward through the transparent portion of the cell measures the absorbance as the rotor is spinning.

______(8) These analyzers have been especially useful for kinetic and enzyme measurements and for end-point analysis as well.

______(9) It is capable of processing 97 tests or samples per hour with a dwell time of 8 minutes, and any combination of tests can be run on any sample at any time.

______(10) Packs for certain tests contain individual disposable chromatographic columns such as ion exchange or gel filtration for removal of interfering substances, such as proteins.

______(11) A built-in computer system reads a binary code for a particular test. The pack header contains these codes.

______(12) Instruments well capable of stat testing.

______(13) Use the principle of measuring the difference in absorbance at two selected wavelengths as a measure of the concentrations called biochromatic photometric measurement.

______(14) A discrete batch analyzer that can process up to 120 test per hour; dwell time is from 1 to 10 minutes; has stat testing capability.

Column A

______(15) A discrete selective analyzer having a throughput of 180 tests per hour and uses a single photometer that measures multiple cuvettes at several wavelengths and multiple times.

______(16) A discrete selective analyzer that processes the high level volume tests such as glucose, BUN, creatinine, sodium, potassium, chloride, and carbon dioxide.

______(17) Will process up to 72 samples per hour and up to 649 tests per hour. Other samples that can be added include amylase, total bilirubin, calcium, and total protein.

______(18) A continuous-flow analyzer.

071. Cite major applications of computers in the clinical laboratory.

Computer Applications in the Laboratory. A major clinical laboratory application of computers since 1970 has been instrument control. For example, the ACA, introduced in 1971, was one of the first of this type of computer. In the past few years, most new instruments that have been introduced contain a microprocessor or a full-fledged computer. To a great extent, these new instruments have been successful because they manage many functions, such as instrument operation, data collection, and processing, all at the same time.

In contrast, laboratory information systems (LIS), the other major laboratory application, have been installed in many larger laboratories.

Computers serve best in collection, distribution, and manipulation of complex or extensive information. The major uses of computers include the following:

a. To perform repetitive tasks.

b. To perform complex calculations rapidly.

c. To collect, organize, store, and distribute large amounts of information.

d. To operate machines in a highly reproducible manner.

Laboratory instrumentation. Since the early 1970's a significant number of instruments introduced into the clinical laboratory are controlled by computers or microprocessors. These "intelligent" instruments include spectrophotometers, chromatographic systems, large discrete analyzers, and large multichannel analyzers. The computer controls each step in many instruments, such as aspiration, of sample and reagents, collection of data, calculation, and printout results.

Newer spectrophotometers with microprocessors are examples of simple computer-controlled instruments.

Operations that are controlled in these instruments include the wavelength scan; the intervals at which observations are made; collection of data; data manipulations, such as subtraction of a previous blank or comparison with a previous scan; and output of the data, either by transmission to an interfaced computer on printout of results, either by a graph or listing.

The Du Pont ACA-III (E. I. du Pont De Nemours, Inc., Wilmington, Delaware) is a prime example of how a computer has been incorporated into a discrete analyzer. Significantly, all the manual operations previously performed for the ACA-I and ACA-II have been automated.

Quality Control. A very significant and major role of laboratory computers is to collect and monitor quality control (QC) data. The application becomes natural because of the ease with which large amounts of data are collected and manipulated in the various calculations.

In some instances the computer is interfaced to the laboratory instrument so that the QC data and patient results may be easily collected. The computer then stores the QC data for later use. For the simplest programs the computer simply compares the current value with the acceptable range supplied by the user. If the result is outside this range, it is flagged so that the user may choose to reject the run.

Information Systems. Laboratory information systems (LIS) are sophisticated computer systems designed to handle the problems of collecting, organizing, and reporting data from the clinical laboratory.

Exercises (071):

1. What has been the major application of computers in the clinical laboratory since 1970?

2. Why are the new automated instruments in the clinical laboratory considered to be successful?

3. What is the other major application of computers in the clinical laboratory?

4. What are some major uses of computers in the clinical laboratory?

5. What are some of the steps controlled by the computer in the "intelligent" instruments?

6. What instrument is cited as a prime example of how a computer has been incorporated into a discrete analyzer?

7. How does the computer operate for the simplest processing and storage of QC data in laboratory instruments?

8. What purpose do the laboratory information systems serve?

CHAPTER 8

Collection and Handling of Specimens—Quality Control

ACCURACY IS BORN when you, the laboratory technician, insure that the appropriate specimen is collected. Thus, quality control in the clinical laboratory begins before the sample is collected from the patient. In most cases, it is your responsibility to secure the correct type of specimen, or at least provide instructions for collecting it.

Blood collection requires a skillful and professional attitude. Your primary objective is to collect the correct type of specimen. Even though the problem of sample collection and processing is a complex one with frequent collection of the inappropriate type of specimens, you can be aware of the varied methods of collections and factors that influence the type of specimen collected. Thus, it is important to determine the type of specimen required for a particular test before you begin to obtain a specimen.

Your laboratory should remind the hospital staff and patients as to the proper method of collecting and storage of not only blood specimen but urine and stool samples for clinical analysis. In addition, your alertness in detecting conspicuous abnormalities—for example, jaundiced serum, hematuria, and lipemia—can be an invaluable diagnostic aid.

Remember, that you, the phlebotomist, are frequently the only representative of the team of highly skilled laboratory workers whom the patient may have the opportunity to observe. The patient is apt to judge the laboratory's performance on the basis of this encounter.

This chapter discusses the collection and handling of specimens and briefly reviews collection variables. The final section deals with the essentials of quality control and the quality control program in the clinical chemistry.

8-1. Collection and Preservation

Collection, processing, and preparation of the specimen prior to analysis must receive prime consideration. If your laboratory results are to have high validity, your technique, including proper manipulation of equipment, use of reagents of specified purity, and correct anticoagulant, if required, must be correct.

072. Specify the reason for selecting a specific vein for venipuncture and the site selection factors; cite patients with the most "difficult veins," and resolve problems when "difficult veins" are encountered.

Review of Venipuncture Techniques. The use of venous blood is absolutely necessary for most tests that require anticoagulants or large quantities of blood, plasma, or serum.

Site selection. For most venipuncture procedures on adults, veins located in the arm are used. Although other veins may be chosen, venous blood is usually obtained from one of the cubital fossa veins as shown in figure 8-1. The median cubital vein is the one most often used because it is large, close to the skin, and the least painful for the patient. One of the cephalic or basilic veins may be used if the venipuncture of the median cubital vein is unsuccessful.

You should consult the nurse or physician before attempting to draw blood from ankle or foot vein sites. These sites are not to be used in patients with diabetes or cardiovascular or circulatory problem.

Site selection factors. Remember that we are attempting to maintain quality control from the initial step in obtaining blood specimens for chemical and other types of analyses; thus, factors in selecting the site should be considered.

Extensive scarring. Avoid all healed burn areas.

Mastectomy (the surgical removal of breast and surrounding tissue). Due to lymphostasis (stoppage of lymph flow), specimens taken from the side on which a mastectomy has been performed may not be truly representative specimens.

Hematoma. Erroneous test results may be obtained from specimens collected from a hematoma area.

Intravenous therapy. Blood should not be drawn from above an intravenous infusion (i.v.) site because the specimen will be diluted with the fluid being administered. Test results from this blood will be erroneous and thus misleading to the physician.

Occasionally i.v. lines will be running in both arms and no site can be found except in the area where the i.v. is being administered. Satisfactory samples may be obtained below the i.v. site if the following procedure is followed:

a. If possible, ask the nurse to turn off the i.v. line; all other nursing personnel must be aware of this action.

b. After 2 minutes, apply a tourniquet below the i.v. site. Select a vein other than the one with the i.v. line.

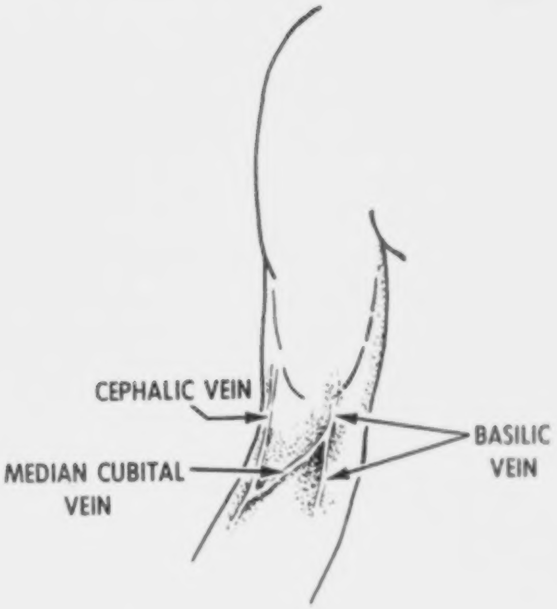


Figure 8-1. Veins of the antecubital fossa.

c. Perform venipuncture. Obtain 5 ml of blood and do not use this specimen.

d. Draw test sample. Apply a firm—but not tight—bandage after removing the needle.

e. Ask the nurse to restart the i.v. line.

f. Note the action taken on the laboratory request.

Techniques to use for difficult veins. Problems in blood collection are encountered with patients who have "difficult veins." The following types of patients may have poor veins.

- Oncology (cancer – related diseases) patients, especially those receiving intravenous chemotherapy.
- Leukemia patients who have had frequent blood tests.
- Patients with constant intravenous therapy.
- Extremely obese patients.
- Babies and children.
- Cardiac patients.

Several techniques are useful when encountering a patient with difficult veins.

a. Look for a blood drawing site: complete forearm; wrists and hands; and ankles and feet.

b. Feel for a vein using the tip of the finger because it is more sensitive. Learn to trust the sense of touch. When feeling for a vein, bear four things in mind: bounce; direction of vein; size of needle; and depth.

Remember, veins will be more prominent and easier to enter when the patient forms a fist. Vigorous hand exercise "pumping" should be avoided because it may affect some values.

The specialist's or technician's index finger should palpate and trace the path of the vein several times as shown in figure 8-2.

c. Choose the veins that feel fullest. Look at both arms. Always feel for the median cubital vein first; it is usually bigger, anchored better, and bruises less. The cephalic vein is the second choice over the basilic vein because it does not roll and bruise as easily even though it flows more slowly.

Exercises (072):

1. Which vein is most often used for venipuncture? Why?

2. Why is it recommended that you consult the nurse or physician before attempting to draw blood from ankle or foot vein sites?

3. Why would a specimen drawn from the side on which a mastectomy has been performed not be suitable for a complete blood count?

4. Why should a specimen for chemistry tests not be drawn from above the i.v. infusion site?

5. When i.v. lines are running in both arms, and no site can be found except where the i.v. is being administered, where can satisfactory samples be obtained?

6. What types of patients may have "difficult veins"?

7. When feeling for a vein, what four things should you bear in mind?

8. After you have looked at both arms in attempting to find a vein, what vein should you feel for first, and why?

073. Cite the reason for the common use of serum rather than whole blood in chemistry testing and relate the significance of using the type of specimen for a given test.

Types of Specimens. Most testing is performed on serum or plasma. The concentration of constituents in erythrocytes is different and usually of less interest. The serum or plasma concentration could remain constant, yet the concentration of whole blood appear to vary, depending on the proportion of erythrocytes in the whole blood.

Serum and plasma. Today there are many methods that use serum or plasma directly without the preparation of a filtrate; such methods are most commonly used in automated instruments. For most analyses, the concentrations of constituents of physiologic or clinical interest are found in the serum or plasma. Since most enzyme determinations require serum, the use of serum for other determinations enables you to run more determinations on the same sample.

Whole blood. There are a few exceptions in which whole blood is preferable.

Glucose-6-phosphate dehydrogenase, galactose-1-phosphate uridyl transferase, and erythrocyte cholinesterase. The main substance of interest is to be found chiefly in the red cells. Heparinized whole blood is used. In such determination, the cells are separated from the plasma, lysed, and the hemolysate used for analysis.

PCO_2 and pH. Heparinized whole blood is preferable. For the determinations of hemoglobin, methemoglobin, and oxygen saturation, whole blood is required.

Carbon monoxide. Only whole blood samples are suitable for analysis because other common fluids such as serum, plasma, or urine do not contain enough hemoglobin to retain a significant quantity of carbon monoxide. Carboxyhemoglobin is stable for at least 2 weeks at room temperature with fluoride as anticoagulant.

Lactic and pyruvic acids. In these determinations, a protein-free filtrate may be made from the blood immediately after collection. Even the short time required to separate the cells from the serum may result in marked changes in the concentrations of these substances.

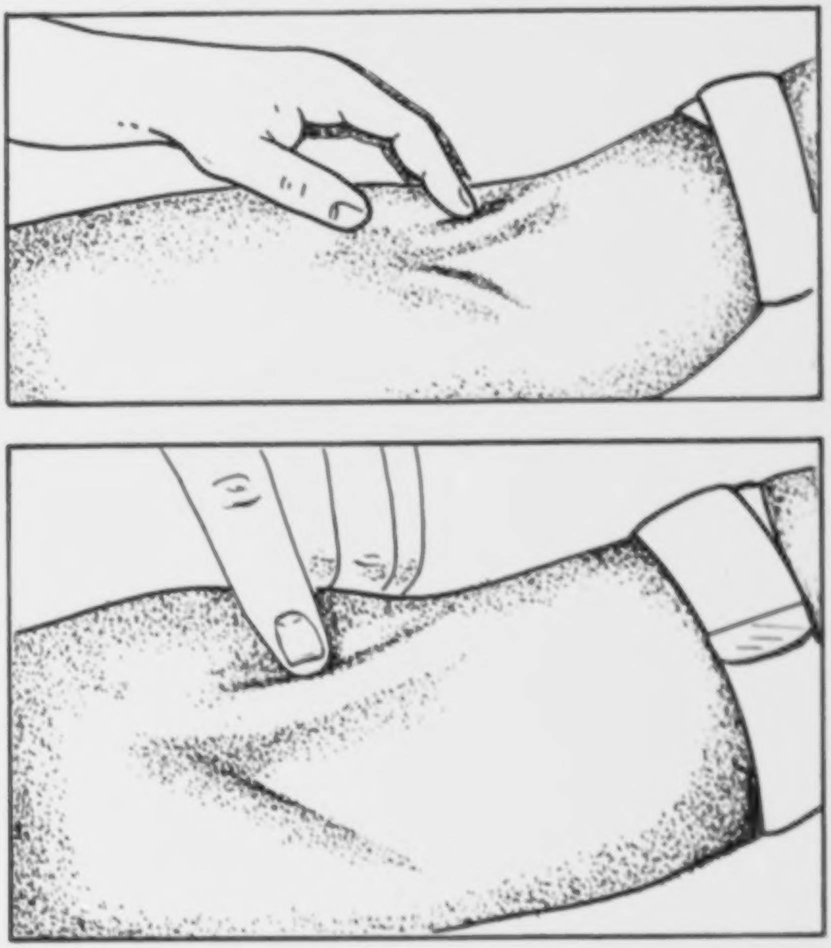


Figure 8-2. Index finger tracing the path of the vein.

Exercises (073):

1. Why would serum or plasma be more commonly used for chemistry testing rather than whole blood?

2. What type of blood specimen would you obtain for the cholinesterase procedure? Why?

3. What type of specimen is required for carbon monoxide determination?

4. What type of anticoagulant is used when stability of carboxyhemoglobin for 2 weeks is expected in the specimen?

074. Specify the characteristics of anticoagulants in terms of their function, purpose, and effects upon cells relative to water loss and gain and upon other constituents for analysis.

Anticoagulants. Whole blood or plasma may be required for some tests. It is necessary that you use the correct anticoagulant to prevent clotting. Not all anticoagulants are equally satisfactory for each test. In preparing plasma, a number of anticoagulants are available. With the exception of heparin, all the anticoagulants function by chelating calcium. The calcium chelating (binding metal ions by forming two or more coordinate bonds with cations) anticoagulants vary in the molarity necessary for effective anticoagulation and in the ability of the anticoagulant to cause water loss or gain from the cells with its associated change in plasma components. For example, the use of the sodium or potassium salts of citrate or oxalate causes a loss of cell water and dilution of plasma, while the use of the ammonium salt causes gain in water by the cell.

A mixture of the potassium and ammonium salts produces a minimum change in plasma or cell water. In contrast, the potassium and sodium salts of ethylenediamine tetraacetate (EDTA) generally do not influence cell water unless the collection tube is only partially filled. As with citrate or oxalate, the use of the ammonium salt of EDTAS leads to an increased cell volume and an increase in concentration of protein fractions in plasma.

Heparin inhibits thrombin and other stages of clotting factor activation. It is in the form of a lithium salt and is an effective anticoagulant in small quantities without significant effect on many determinations. It is the ideal universal anticoagulant for blood from which plasma may be obtained. Heparin is generally the preferred anticoagulant when plasma is required for chemical testing. If heparinized plasma has to be stored, it should be centrifuged before assay, since small fibrin clots frequently form in heparinized samples following storage. In addition, it is often quite convenient and timesaving to use a cell-separating aid to help separate plasm or serum from cells.

Sodium fluoride is an effective anticoagulant because it removes calcium, but because of its relative high concentration it produces water shifts and is also likely to produce hemolysis. Fluorides are preservatives through their inhibitory actions on certain enzymes such as those involved in glycolysis. Fluoride will inhibit the action of glucose oxidase in the enzymatic analytical method for glucose, will diminish the activity of acid phosphates, and will increase the activity of amylase.

Exercises (074):

1. With the exception of heparin, how do all anticoagulants function?

2. By what two characteristics do the calcium chelating anticoagulants vary?

3. How does sodium or potassium salt of citrate or oxalate differ from ammonium salt by their effects on the cell, water, and plasma dilution?

4. What mixture produces a minimum change in plasma or cell water?

5. What anticoagulant does not influence cell water?

6. How does heparin prevent coagulation?

7. What anticoagulant is generally preferred when plasma is required for chemical testing?

8. How do fluorides act as preservatives?

9. What effects would you expect on a glucose result if plasma was used from fluoride tube and the glucose oxidase method was used for analysis? Why?

075. Cite some variables in sample collection that affect given test results and state why these effects occur.

Variables in Sample Collection. Some factors do significantly affect the results of laboratory tests. Thus, when unexpected values are observed, you should be aware of these possibilities.

Daytime variation. Some tests show significant variation when samples are collected during the day. Some examples of these are: Cortisol, Iron, Estriol, Catecholamines, Corticosteriods, Glucose, and Triglycerides.

Variation within a day for these substances may be as much as 30 to 50 percent. It is best to collect samples as soon as the patient wakens.

Posture. The posture of the patient at the time of collection can have a significant effect upon protein and protein-bound substances in the serum. Some components in serum known to demonstrate postural variation are the following: Total protein, albumin, lipids, iron, calcium, and enzymes.

When patients move from the supine to the standing position, the serum concentration of these constituents increase 5 to 15 percent. The movement of water out of the intravascular compartment upon standing probably causes this effect. The effect may be minimized by (1) drawing all blood samples from supine patients and (2) establishing reference ranges using blood drawn from supine, healthy volunteers.

Stasis. A number of laboratory test results may be elevated by the prolonged use of a tourniquet. If your tourniquet is applied for an extended period, stasis or pooling of blood above the constriction will result. Prolonged stasis causes false elevation of results.

Hemolysis. Care must be taken to minimize hemolysis during sample collection. Hemolysis may result because of the use of too large or small a needle, moisture in a syringe, vigorous mixing of blood, rapid expansion of the blood in the tube, or the separation process. Regardless of the cause, the end result is a false increase in serum concentration of constituents that are present in high concentrations within the red blood cells. Conversely, for those substances that exist at lower concentrations in the red cells than outside, hemolysis will result in a dilution effect on the serum constituents.

Common constituents whose concentrations are significantly affected by hemolysis are:

Total protein
Albumin
Lipids
Iron
Calcium
Triglyceride
Norepinephrine
Renin
Aldosterone
Potassium
Enzymes
Bilirubin
Cholesterol
Magnesium
Inorganic phosphorus

Visible hemolysis is not necessary for this phenomenon to take place and may result from prolonged cell-serum contact when the serum is not promptly separated from the cells. Leaking of potassium and enzymes may occur without the visible leakage of hemoglobin.

Stress of phlebotomy. The stress of phlebotomy may also affect laboratory results. Catecholamine level and blood-gas results can be affected through direct hormonal effects and hyperventilation caused by anxiety of the patient. You should make every possible effort to insure that the patient is calm before collecting the sample. Also, during phlebotomy, errors may arise from improper swabbing of the venipuncture site, causing trauma to the arm or drawing too close to the site of the intravenous infusion.

Exercises (075):

1. What are some tests that will show daytime variations as much as 30 to 50 percent?

2. What are some test that are affected by postural variation?

3. What causes a change or variation in serum constituents to increase from 10 to 15 percent when patients move from the supine to the standing position?

4. What is stasis and how is it caused?

5. What are some causes of hemolysis?

6. What are two possible effects of hemolysis on serum constituents?

7. What two given constituents may leak from the red cells without visible leakage of hemoglobin?

8. What test results may be affected by direct hormonal effects and hyperventilation?

076. Cite conditions that require drawing blood specimens from patients in a fasting state and specify requirements for accurate preparation, stability, storage, referral, and shipment of specimens for chemical analyses.

Variation in Patient Preparation. The widespread practice of drawing blood specimens from patients in the fasting state appears more difficult to explain from a technical standpoint than from a standpoint of administrative convenience.

Laboratory personnel ordinarily use the fasting requirement in scheduling patients simply because of this administrative convenience. However, with the exception of glucose, triglycerides, and inorganic phosphorus, most chemical substances reveal no significant changes after a standard breakfast. Certainly the pertinence to particular test must be considered, especially when a cloudy serum is obtained. Some difficulty has been reported with uric acid determinations, and cholesterol is reported to be elevated following a meal that is high in cholesterol. Some chromogens present in food may produce icteric serum, and postprandial glucose will lower phosphorus values. If you do not know the possible effects of eating upon a particular test, you may feel safer in requiring a fasting specimen. Although it has been recommended that you instruct the patient to fast before drawing the sample to insure that laboratory results are compatible with "reference values," prolonged fasting for more than 24 hours can lead to unexpected laboratory results. Prolonged fasting has been associated with elevations in serum bilirubin concentration as well as decreases in the plasma concentrations of glucose and proteins.

Some tests require the patient to fast or to eliminate certain foods from the diet before drawing. Time and diet vary according to the test. Such restrictions are needed to insure accurate test results. Therefore, as a matter of general policy, it is advisable that patients fast for at least 12 hours before blood collection for chemical analysis.

Specimen Stability and Storage. After the specimen is obtained, care must be taken to maintain the integrity of its constituents.

Whole blood. The blood should be kept in the original container until ready for analysis, which should begin within 1 hour after drawing blood. Store the blood in a refrigerator at 4°C to 6°C. Do not freeze. If a filtrate is to be used, prepare at once and refrigerate.

Plasma. The blood should be centrifuged for at least 10 minutes within 1 hour after collection (in the original container), keeping the container stoppered to prevent evaporation. The plasma container with the specimen must be labeled and stored in a refrigerator at 4°C to 6°C until the plasma is analyzed, or frozen at −20°C if analysis is delayed more than 4 hours.

Serum. To process blood for serum, the blood should be allowed to clot in the original closed container at room temperature. When the clot has formed, gently loosen it at the tip with an applicator stick if necessary. Centrifuge the blood for 10 minutes in the stoppered container. Unless otherwise specified, label and store the serum in a refrigerator at 4° to 6°C until analyzed, or freeze at −20°C if analysis will be delayed more than 4 hours. There has been a recent report that placing of wood applicator sticks in serum for extended periods of time may produce analytical artifacts. Potassium, calcium, and glucose concentrations increase after only 3 minutes of contact with wood applicator sticks.

Serum-separator devices. Many types of serum-separator devices are commercially available for safe and easy use. There are two kinds available, those used during centrifugation and those used after centrifugation. Devices used during centrifugation may be either integrated gel-tube systems or devices inserted into the collection tube just before centrifugation. Integrated gel-tube systems contain a gel that starts at the bottom of the tube. During centrifugation, due to its viscosity and density, the gel floats to a position above the cells and below the serum. The need to remove the stopper before centrifugation is avoided and aerosol production and evaporation do not occur, thus saving time.

There are devices that may be added after centrifugation to separate the clot from serum. Usually, they are plunger-type filters, having a plastic tube with a filter tip at the end in a plastic or rubber base. When centrifugation of samples is completed, these devices are inserted with the filters passing through the serum, stopping just above the surface of the cell avoiding contact with the cells that can cause hemolysis. the device is then withdrawn slightly to produce a small air gap below the filter, separating the filter from the cells. This reduces the potential for leakage of constituents from the cells through the filter into the serum.

Specimen Referral and Shipment. Area laboratories generally establish their own requirements for referral specimens. Whenever a specimen is referred to another laboratory, it should be labeled with all of the data necessary to identify it, the date drawn, and preparation details. For example, if a preservative has been added, the container should be labeled with this information. There is sometimes a problem in the stability of certain specimens which may prevent their being referred to another laboratory. If transportation requires time in excess of that which is known to cause deterioration, there is no point in shipping clinical material just to secure some type of report. It is desirable for the performing laboratory to maintain control of specimens to insure that improper handling or storage has not invalidated results. This is not always possible, and it then becomes the responsibility of the referring laboratory to handle materials with a knowledge of chemical principles and requirements of the test requested. If blood, urine, and other substances must be shipped to another laboratory, they must be shipped promptly and in good condition.

Exercises (076):

1. What chemical constituent will be lower after a postprandial glucose?

2. What effect does prolonged fasting for more than 24 hours have on serum bilirubin? Glucose and proteins?

3. A general policy of having patients fast for at least 12 hours is a type of restriction to insure ____________ ____________ ____________.

4. Unless otherwise specified, how should serum be processed after centrifugation and removal from the clot?

5. What constituents have shown an increase in concentration after only 3 minutes of contact with wood applicator sticks?

6. When using the plunger-type filter to separate serum from the clot, why is the device withdrawn slightly to produce a small air gap below the filter, separating the filter from the cells?

7. What are some requirements when a specimen is referred to another laboratory?

077. Cite procedures for processing and handling cerebrospinal fluid in the clinical laboratory.

Handling CSF in the Laboratory. Spinal fluid is more difficult to obtain than most other specimens; this, consequently, places more responsibilities on the laboratory as far as proper handling of specimens is concerned. It is often stressed that spinal fluid is highly dangerous material as a potential source of contagion to the laboratory technician. This popular concept is not entirely wrong, nor is it entirely correct, because it overemphasizes the dangers of handling cerebrospinal fluid (CSF). Spinal fluid is a source of infection, just as any other body fluid. Perhaps a general lack of familiarity with CSF is responsible for people maximizing its danger and treating it more cautiously than they do blood or urine. Rather than stress the highly infectious nature of spinal fluid, it is more appropriate to stress proper handling to avoid errors or loss of the sample.

The entire reservoir of CSF in the body rarely exceeds 150 ml. The physician seldom obtains more than 8 or 10 ml. Usually the physician obtains much less; therefore, none can be wasted. A physician usually submits spinal fluid to the laboratory in three tubes numbered consecutively as they are drawn. The tubes are numbered to distinguish the initial tube (which is most often contaminated with blood from the trauma of the spinal tap) from the second and third tubes. The second tube contains less cellular contamination, and the third tube is for cellular analysis of the spinal fluid. If vials come directly from the patient's room or from surgery, they may not be properly labeled. In this case, the first thing you should do is label the tubes. It has become generally accepted that tube number 2 is used for chemistry determinations. Other tubes may also be used for chemistry, if they are free of blood.

The possibility of centrifuging cells from a bloody tube and using the supernatant for chemistries is not to be excluded. But it is not usually considered acceptable to do so when the purpose is to remove a significant amount of blood from a traumatic puncture. If a tube contains blood, it follows that serum will remain in the supernatant fluid after centrifugation. This fluid could not be used for protein determinations, and if the amount of blood is significant, it probably could not be used for other tests.

Cloudy spinal fluid is always centrifuged before chemical analysis, but for a different reason. Most constituents of normal spinal fluid are represented in lower concentrations than they are in blood (an exception is chloride, which is present in greater concentrations in spinal fluid than in serum). Hence, for most tests, serum will elevate the constituent being measured.

The amount of blood present and the nature of the test could be used to decide whether or not a particular determination is valid. Less than ideal CSF specimens (icteric, hemolyzed, etc.) should not be indiscriminately discarded without first consulting the physician regarding the urgency of the case. Although the clinical laboratory does not control actual drawing of the specimen, you should do everything possible to aid the physician.

It will be rare, indeed, that you will be in a position to keep spinal fluid for any length of time. Tests on spinal fluid are usually ordered on an emergency basis, or at least with the intent of receiving the results within a matter of hours. Refrigeration is generally adequate for limited preservation, except in the case of spinal fluid glucose. If bacteria are present, a spinal fluid glucose will drop markedly in a few hours due to bacteria utilizing the glucose in their metabolism. As stated before, cloudy spinal fluid should always be centrifuged. But this will not eliminate bacterial contamination, and the specimen cannot be saved as it could have been if it had been sterile.

Exercises (077):

1. What is the first thing that should be done when the laboratory receives CSF tubes?

2. Which of the three tubes submitted to the laboratory for spinal fluid analysis is used for chemistries?

3. Why is a tube containing blood not used for protein determinations?

4. What constituent is usually higher in CSF than in serum?

5. How will the presence of bacteria affect the spinal fluid glucose?

8-2. Quality Control

Standards and precalibrated curves provide a means of calculating the value of an unknown specimen. However, calculation of a result does not insure the quality of that value. There may be a variety of factors which tend to invalidate the test results. There are always problems of reagent instability, contaminated glassware, instrument variation, and technician error. Even the most careful and experienced technician is not infallible and is subject to certain physical limitations. Unless the doctor knows the precision of a result, he or she is not justified in accepting it as a basis for diagnosis or treatment. Yet, it is not possible for a doctor to analyze every item of laboratory data for accuracy, and indeed that is not his or her responsibility. Rather, you, as the laboratory technician, must control the quality of your work within certain established limits.

Quality control is an attempt to insure reliability of the tests performed. What possible good is a test if the result is inaccurate? Unrealiable results will mislead the doctor and quite possibly harm the patient. Both you and the doctor know there is always a degree of uncertainty with each result obtained. This is where quality control comes in. It tells us the degree of uncertainty which always goes with a result. The object of a good quality control program is to keep this degree of uncertainty within narrow limits.

As is the case in industry and in other sciences, the clinical laboratory has adapted certain statistical procedures which control the accuracy of results. All data submitted by the laboratory should be substantiated by sufficient statistics to prove that it is within reliable limits. It must be accepted that the worth of a laboratory rests on its proficiency and the quality of its work. There is no substitute for statistical proof of a result. As is true in any science, terms used most have exact meanings, and the language is principally one of mathematics. In this section you will have an opportunity to review the necessary elements of a quality control program.

078. Define specific factors related to a chemistry standard.

Standards. As indicated earlier, a good quality control program keeps the degree of uncertainty within narrow limits. However, there is often confusion in the use of the terms "standard" and "control." A standard is a sample of exact known composition that is used to calculate the result of a sample of unknown composition. A prepared curve is a graphic representation of a standard of series of standard results.

The value of a standard must obviously meet two basic requirements. First, it must be exact. A range is not suitable if the calculation is to result in an exact figure. For example, a common ruler may be considered a standard. If it s used to measure the top of a desk, the result is expressed in terms of the known length of the ruler, in units such as inches. Suppose you measure the desk at 3 ruler lengths, or 36 inches. Suppose the ruler is not exactly 12 inches long, but approximately 10 to 14 inches. The calculated result would also be approximate; the desk you measured could actually be 30 to 42 inches long. You can readily see why a standard must be exact.

Second, the standard value must be known. There should never be a question in your mind as to the value of the standard. If you think that your ruler is 12 inches long but do not know this for certain, the measurement is also uncertain. Your answer should be expressed in a number of significant figures commensurate with calculations and procedures involved in preparation of the standard and in the procedure itself. To expand the example of a ruler, you would not expect to measure an item to the nearest 10th of an inch with a ruler that was not graduated in 10ths of an inch. It is not always necessary to run a chemical standard through all steps of a test. For instance, the final color produced may be compared with a color chart or colored solution which would be considered the standard. A standard is frequently a pure chemical in a suitable solvent and does not usually contain extraneous material.

Chemical Purity. You have worked with many chemicals in the clinical laboratory. No doubt you realize that there are degrees of chemical purity, each with its own variations. Perhaps we should clarify what is understood by the term "chemical purity." We refer to A.C.S. grade chemicals, U.S.P., N.F., technical, and reagent grades. In addition, there are specific purity requirements for particular chemicals used in specific tests. These terms are often confused, so let us first define them.

A.C.S. (American Chemical Society) grade. This is the only universally accepted standard of chemical purity. The A.C.S. designation is assigned if a chemical assay passes American Chemical Society specifications for purity.

U.S.P. (United States Pharmacopeia) grade. Ths designation was assigned by the U.S. Pharmacopeia Convention primarily on the basis of therapeutic value. U.S.P. chemicals usually are not of sufficient chemical purity for use as reagents in clinical chemistry.

N.F. (National Formulary) grade. This is also a grade designation assigned on the basis of therapeutic value, and this is useful in pharmacology. N.F. grade chemicals are not pure enough for general use in clinical chemistry reagents either. This designation is assigned by the Committee on National Formulary by authority of the American Pharmaceutical Association.

Technical grade. These chemicals are manufactured primarily for industrial use. This grade of chemicals is of reasonable purity for commercial processes but unsuitable for use in quantitative chemistry because of contaminants.

Reagent grade. These are chemicals manufactured for use as reagents. This designation is assigned to various chemicals by individual chemical companies, and the designation may or may not meet specific reagent requirements. In some cases, a company may certify a certain chemical or reagent for use in a specific method of analysis. This certification is usually valid when it is made by a reliable manufacturer. It always relates to a particular test and does not imply chemical purity other than the minimum allowable in the specific test procedure. Generally, published methods state the chemical purity limitations when they are a factor in the reaction. If the requirements are not stated, it is assumed that the most pure chemical available will be used.

Exercises (078):

1. Match each standard, control, or grade of chemical purity in column B with the correct definition or descriptive statement in column A. Each column B item may be used once or more than once.

Column A

_____ (1) May be certified by a company for use in a specific method of analysis.
_____ (2) Its value must be exact and known.
_____ (3) Assigned designation on the basis of therapeutic value.
_____ (4) Useful in pharmacology.
_____ (5) Unsuitable for use in quantitative chemistry because of contaminants.
_____ (6) Frequently a pure chemical in a suitable solvent and does not usually contain extraneous material.
_____ (7) The only universally accepted standard of chemical purity.
_____ (8) Manufactured primarily for individual use.

Column B

a. Standard.
b. A.C.S. grade.
c. U.S.P. grade.
d. N.F. grade.
e. Technical-grade chemicals.
f. Reagent-grade chemicals.

079. Compare primary and secondary standards and cite differences between standards and controls.

Types of Standards. The two basic types of standards are (1) primary standards and (2) secondary standards.

Primary standards. Primary standards consist of the purest form of a chemical substance dissolved in a suitable solvent. The lack of contaminants in a primary standard is not completely desirable, however, because a pure standard does not react entirely as a test specimen which contains inhibitors or other contaminants. Some primary standards are available commercially which contain extraneous substances, but meet the basic definition of a standard in containing a pure, weighed quantity of the chemical to be assayed.

Secondary standards. Secondary standards are not chemically identical to the substance assayed, but they are related through some physical or chemical property which makes them useful as standards. An ordinary color comparison chart could be considered a secondary standard.

Use of Standards. It should be reiterated that use of a standard in no way verifies or controls a result. An accurate standard may be used in a manner which results in completely unreliable results. For example, if a miscalibrated spectrophotometer is used, the quality of the standard will be invalidated even though the standard is most carefully prepared. The same is true of a prepared curve, with the added danger that a curve that is not checked each time the procedure is performed may not hold under conditions that have changed since the curve was established.

Controls. A control is a specimen of known approximate value which is treated in the same way as the unknown specimen. The range or value for a control ideally should be close to that of the specimen being assayed. For example, a group of unknown bilirubin samples with values near 15 mg/dl should be run with a control near 15 mg/dl. One reason is that a control of lower value would not prove that a high result could be achieved.

Differences Between Controls and Standards. A control obviously differs from a standard in various ways. Ordinarily, control serum has properties similar to the properties of the unknown specimen. It may contain protein and other constituents which are present with the unknown.

Further, a control is not necessarily assigned a definite value, and the constituent being controlled may not have been accurately weighed. Where the constituents of a control specimen have been accurately weighed and dissolved in a suitable solvent, the concentrations given are referred to as "weighed-in" values. However, if the concentrations of constituents are determined from pooled samples of unknown concentration, the values given for the control serum are called "assayed" values. Both values depend upon the percent recovery of the specific assay method used. Therefore, some notice should be taken of the method used to quantitate constituents of the standard. Ideally, the assay method should be the same as the method you wish to control. This is not always possible, but information can be obtained by comparing the confidence limits of different methods.

There should be no doubt that standards and controls have different purposes and cannot be interchanged. If a calculation is based on the value of a control, the control does not automatically become a suitable standard. A solution that does not contain an exact known amount of the constituent in a suitable solvent falls short of the definition reserved for a standard.

Exercises (079):

Indicate whether each of the following statements is true (T) f false (F). If you indicate "false," explain your answer.

_____ 1. Primary standards consist of the purest form of chemical substance dissolved in a suitable solvent.

_____ 2. Lack of contaminants in a primary standard is completely desirable.

_____ 3. Some primary standards may contain extraneous substances even though they meet the basic definition of a standard, containing a pure, weighed quantity.

_____ 4. Secondary standards are chemically identical to the substance assayed.

_____ 5. An ordinary color comparison chart could be considered a secondary standard.

_____ 6. The use of standard will verify or control the result of a test.

_____ 7. If a miscalibrated spectrophotometer is used, the quality of the standard is unaffected.

_____ 8. It is not necessary to check a prepared curve frequently with a standard since the curve is stable.

_____ 9. A control is a specimen of known approximate value which is treated the same way as the unknown specimen.

_____ 10. The range of the control should be either much higher or much lower than the specimen being assayed.

_____ 11. A control is always assigned a definite value, and the constituent being controlled has been accurately weighed.

_____ 12. When concentrations of constituents are determined from pooled samples, the values in the control serum are "assayed" values.

_____ 13. Standards and controls have different purposes and cannot be interchanged.

_____ 14. If a calculation is based on the value of a control, the control automatically becomes a suitable standard.

080. Identify correct terms, examples, and procedures with good and poor quality control.

Accuracy, Precision, and Validity. If you achieve a value that is within the allowable variation established for a particular determination, it is considered *accurate*. It is another matter to consistently achieve accurate results. The concept of duplicating a result within the allowable range in two or more determinations is termed "precision" (reproducibility).

It is emphasized that accuracy and precision are not synonymous. It is possible to achieve accuracy without precision, and it is possible to have precision without accuracy. For example, assume that the actual glucose value of a serum sample is known to be 100 mg/dl. Duplicate determinations yield 80 mg/dl and 120 mg/dl, respectively. The average of 100 mg/dl is indeed accurate, but results are not reproducible. The extent to which a result may vary from the established value is determined statistically, as explained in the following section. In any case, a variation of 40 mg/dl (120 down to 80) for a glucose determination does not appear to be within acceptable limits.

A second possibility is precision without accuracy. Obviously, a laboratory could perform a particular procedure several times and consistently reproduce erroneous results. In the case of the glucose example, a result of 80 mg/dl might be achieved on two or more consecutive determinations. This is not nearly as absurd as it may seem. A laboratory using reagents that are incorrectly prepared or deteriorated could repeatedly obtain inaccurate results. When nonautomated procedures are frequently performed, poor individual technique, on the other hand, most often leads to a loss of precision.

Another term that may not be completely clear to you is "validity." A procedure is considered valid if it measures consistently what it is intended to measure. A procedure for alkaline phosphatase, for example, would not be valid if the pH of the substrate is in a range of 4.8 to 6.0; however, it could well be reproducible.

Problems in Quality Control. A quality control program must be established with some knowledge of problems that can exist and with a working knowledge of exact terminology. The concepts of accuracy and precision encompass a variety of problems that must ultimately be identified. Be certain that you are able to cite an authority or current reference for every step of your procedure. Recognize, too, that references are sometimes in error and must be cross-checked and updated. Some factors responsible for poor quality may be listed as follows:

a. Faulty or inaccurately calibrated equipment, including glassware.

b. Poor technique.

c. Variation in test conditions, such as time and temperature.

d. Dirty glassware.

e. Incorrect curve, standard, or factor.

f. Poor method or method modification without standardizing for accuracy and reproducibility.

g. Unsuitable specimen.

h. Unsatisfactory reagents.

i. Contamination and interference.

j. Errors in observation and calculations.

Exercises (080):

1. Match each value, example, or procedure related to quality control in column A with the term or phrase in column B that is most closely associtated with it.

Column A

_____(1) A value achieved within the allowable variation established for a particular determination.
_____(2) Sample of BUN value: 13 mg/dl; duplicate determinations 10 mg/dl, 15 mg/dl, 15 mg/dl, 10 mg/dl.
_____(3) Sample of potassium value: 4.5 mEq/L; duplicate determinations, 3.5 mEq/L; 3.6 mEq/L, 3.8 mEq/L, 3.7 mEq/L.
_____(4) Duplicating a result within the allowable range in two or more determinations—reproducibility.
_____(5) A procedure measures consistently what it is intended to measure.
_____(6) Ability to cite an authority or current reference for every step of your procedure.
_____(7) Cross-checking and updating references.
_____(8) Use of incorrect curve, standard, or factor.
_____(9) Use of reagents beyond manufacturer's outdates.
_____(10) Frequent periodic validation of established curves.

Column B

a. Accuracy.
b. Precision.
c. Validity.
d. Poor quality control.
e. Good quality control.
f. Accuracy without precision.
g. Precision without accuracy.

8-3. Laboratory Mathematics

A substantial number of errors that occur in clinical chemistry are traceable to errors in mathematics. We have already discussed the necessity of preparing and maintaining records of each procedure. The degree of automation will largely determine the nature of such records. In some cases, there may be little more involved than recording a result. If various mathematical procedures are involved, there is always the possibility of error, even in simple arithmetic. As we stated earlier, science is most scientific when it is expressed mathematically. The technician who excuses his or her lack of facility in mathematics does not change the fact that clinical chemistry is expressed numerically. The ability to state a problem and express a result in mathematical language is invaluable. If practice is necessary, it should be understood that each of us must practice until we achieve proficiency.

081. Define the term "significant figure," cite its use in reporting laboratory test results, and identify the significant figures in given numbers.

Review—Numbers and Significant Figures. One area which relates directly to mathematics and accuracy in reporting results is the concept of significant figures. Technicians sometimes have the tendency to carry mathematical calculations to unnecessary points. This may be the result of desiring to be as accurate as possible, a lack of knowledge as to the precision of the test itself, or just lack of knowledge of significant figures. The proper retention of significant figures in data and in the reported results is an important consideration because the number of significant figures should relay to the physician an approximate indication of the precision of the method.

A *number* is an expression of quantity. A figure or digit is any one of the characteristics 0, 1, 2, to 9, which, either alone or in various combinations of more than one character, serve to express numbers.

A *significant figure* is a digit that denotes the amount of the quantity in the spot in which it stands. Thus, you may further consider any figure that represents an actual measured quantity to be a significant figure. For example:

345 - These figures (digits) state that there are three hundreds, four tens, and five units. They are all significant figures.

045 - Only digits in the tens and units are significant figures.

450 - All three digits are significant.

When the figure 0 is used to located the decimal point, it may or may not be a significant figure. For example:

0.345 - Here the zero is not a significant figure.

0.00345 - Here the zeros are not significant figures, since they merely place the decimal point in its correct position with regard to 3, 4, and 5.

10.510 - Here the zeros are significant, since they state that this number is greater than 10 but not quite 11, and more precisely closer to 10.510 then 10.509 or 10.511. Thus, both zeros are significant in this number.

Significant Figures in Laboratory Results. This information could be applied when reporting test results. For example, a result that is reported as 1 mg per ml or 100 mg/dl is expressed in one significant figure. If it is written as 100.5 mg/dl, there are four significant figures. The key to identifying a figure as significant is to determine whether it represents a measured value. Zero is not significant if it appears as the first figure of a number, as in 0.1; but in the expression 1.0 there are two significant figures. You are encouraged to refer to a textbook of mathematics if the concept of significant figures is not clear.

You will find that it pays to be conscientious in submitting numerical reports in the correct number of significant figures. A glucose result that is reported to the fourth decimal is likely to cast a reflection on the laboratory that produced it. Further, since it is meaningless to report a result with a degree of accuracy that does not exist in the procedure, this should not be attempted.

Exercises (081):

1. What is a significant figure?

2. Which are significant figures in the following examples?

   a. 675.

   b. 067.

   c. 6750.

3. Is the zero in 0.967 a significant figure?

4. In the number 0.00967, are the zeros significant figures?

5. Are the zeros in the number 20.510 significant?

6. How are significant figures for laboratory results reported?

8-4. Establishment of a Quality Control Program

If you have a genuine desire to maintain the highest quality in your work, it is not difficult to establish a quality control program. While the discussion of this type of program is limited in its application to clinical chemistry, a quality control program may be established for any area of the clinical laboratory. This section explains how you can establish such a program.

082. Specify, in terms of related duties, the levels of responsibility for quality control among all laboratory personnel in clinical chemistry.

Quality Control Organization. Your quality control program cannot succeed without the understanding and support of all chemistry personnel. The first line of responsibility in the daily quality control program is the technician performing the test. You, the alert technician, can identify problems before they affect quality control (QC) specimens or patient samples by monitoring all factors associated with standardization and instrument performance. Such factors or parameters are stable, and any change in them could indicate potential problems.

The next line of responsibility is the supervisor. You, as the supervisor, must be aware of problems in areas for which you are responsible and correct them. The duties include writing clearly written procedures for all analyses in order to reduce variation in techniques. You must also provide written instructions concerning steps to take when the QC system indicates a problem and help to analyze trends and choose samples for repeat analysis.

The OIC or director of the clinical laboratory ultimately must insure that problems are identified and that corrective action is taken. Accurate and frequent communication between the technicians, supervisory technicians, and the chemistry OICs or director is essential. The key ingredient of your QC program is that the technicians, chemistry supervisor-technician, and director all be conscious of quality control and their roles in assuring meaningful laboratory data.

Exercises (082):

1. Who has the first line of responsibility in the daily quality control program?

2. How can the alert technician identify problems before they affect QC specimens or patient samples?

3. What is basically the responsibility of the chemistry supervisor?

4. What do some of the supervisor's duties include?

5. What is the responsibility of the OIC or director of the clinical laboratory for QC in clinical chemistry?

083. Cite recommendations for maintenance of reagent quality control, for the use of serum-based controls and calibrators, for daily considerations, and for personal improvement.

Reagent Quality Control. Water is the most commonly used reagent in the chemistry laboratory, and its quality should be routinely monitored. A monitor that should be used is the temperature-corrected specific conductance of the water available at the outlets in the laboratory. Conductivity meters and electrodes are available from a number of sources and should be used at least weekly if a continuous monitoring system is not available. Although measurement of conductivity is the best single measurement of water purity, it is important to check for other substances, depending on the particular use.

When reagents are received as bulk materials, the "date received" should be noted on the container and, when first opened, the "date opened" noted. You an use these commonsense precautions to prevent outdated materials from being used. When reagents are prepared, the minimum labeling requirements are: name of the test for which the reagent was prepared, reagent name, date of preparation with initials of the person who prepared it, date of reagent check with initials, date of expiration, and any special instructions or warnings.

Serum-Based Control. Serum-based control material may be purchased commercially or manufactured by the laboratory. Reliable commercial control serums are readily available in various unit volumes, assayed, unassayed, large or small lots, in normal, or abnormal ranges, and for enzymes or special tests. The cost is nominal when it is considered that reliable chemistry results are as good as the quality control program that supports them.

Calibration. Controls may not be used as calibrators. Controls and calibrators must be different, because each has a separate and important function. Each method of analysis should have a calibration system that is independent of the control system. The calibrator has been assigned a value by the manufacturer or the user by a reference method. The calibrator materials come in a variety of forms such as aqueous and serum matrices. A material used to calibrate may not be used as a control, and vice versa. Calibrators are usually purchased in lots large enough to last 12 to 18 months. Keep in mind that the overall goal is to maintain a year-in, year-out consistent level of analytical accuracy.

Daily Considerations. You should check out new batch lots of commercial reagents as soon as they are received. It is also good practice to schedule maintenance so that you can establish a test set of controls and run a few patient samples from a previous batch before the next regular daily run is processed. You should maintain a record of all solution changes and all instrument repairs. All maintenance procedures must be kept to help in subsequent troubleshooting or in preparing maintenance schedules. It is important for you to know that this documentation is required by the Joint Committee on Accreditation of Hospitals (JCAH) and the College of American Pathologists (CAP) accreditation programs.

Improvement. All laboratory technicians should strive to improve their own status and capabilities by joining appropriate professional societies, reading technical journals and books, attending scientific meetings, and taking continuing education courses.

Exercises (083):

1. What is the best single measurement of water purity in the chemistry laboratory?

2. What information should be noted on the container when reagents are received as bulk materials?

3. What are some minimum labeling requirements when reagents are prepared?

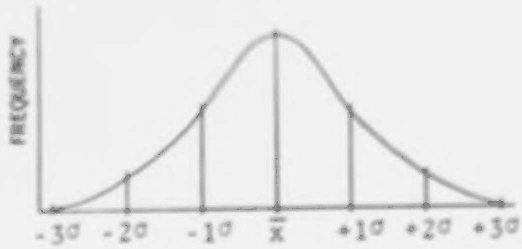


Figure 8-3. Frequency distribution curve.

4. Why is it recommended that serum-based controls not be used as calibrators?

5. What is the purpose of the calibrator?

6. Why are calibrators usually purchased in lots large enough to last 12 to 18 months?

7. When should you check out new batch lots of commercial reagents?

8. What information should a daily record show, relative to solutions and instruments in daily use?

9. What agencies require the documentation of information in exercise 8?

10. What recommendations are made for technicians to improve their status and capabilities?

084. Cite the significance of quality control in its statistical context, and specify the use of the standard deviation for measuring precision in procedures.

Statistical Expression. Quality control implies a system for measuring the degree of precision in procedures. In its statistical context, quality control does not measure the accuracy of a procedure. It does measure variables, including reagent and method reliability, technician skill, and instrumentation. Variables are estimated statistically in an expression of total allowable variation, termed ''confidence limits.'' Confidence limits are established by calculating the dispersion of values for a test on either side of the average value. This mathematical measurement is termed ''standard deviation.'' It is desirable that you have some knowledge of statistical frequency distribution. If a laboratory test is performed on a sufficiently large number of normal people and a distribution curve is plotted of the frequency of results, we would expect to obtain a typical bell-shaped curve. The peak of this curve would be at or near the average of all values obtained. The height of the curve would decrease as we moved away from the average in each direction. A sample curve is shown in figure 8-3. Standard deviation (SD) is calculated from the values that made the curve, by the following formula:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n - 1}}$$

where σ is the standard deviation, Σd^2 is the sum of the squared differences from the average, and n is the number of test results used.

Standard deviation tells us that 68 percent of the values are in an area from the average - 1 SD to the average + 1 SD ($-\sigma$ to $+\sigma$). Likewise, 95.5 percent of the values are between -2σ and $+2\sigma$, and 99.7 percent from -3σ. Usually two standard deviations, which represent 95 percent confidence limits, are used for laboratory work. Many laboratories use ± 2.5 SD, which includes 98.8 percent of the values. This would allow for a possible 12 outlying values for every 1,000 determinations.

How can we apply this to assure accuracy in the laboratory? This will require some effort and at least 3 weeks before practical results can be realized. The first step is to establish a standard deviation for each test you want to control. After this, daily analysis of the control specimen described previously will instantly tell you if your results are within the confidence limits desired.

Exercises (084):

1. What does quality control measure in its statistical context?

2. What is meant by ''confidence limits''?

3. Basically, how are the confidence limits established?

4. How many standard deviations are used for laboratory work?

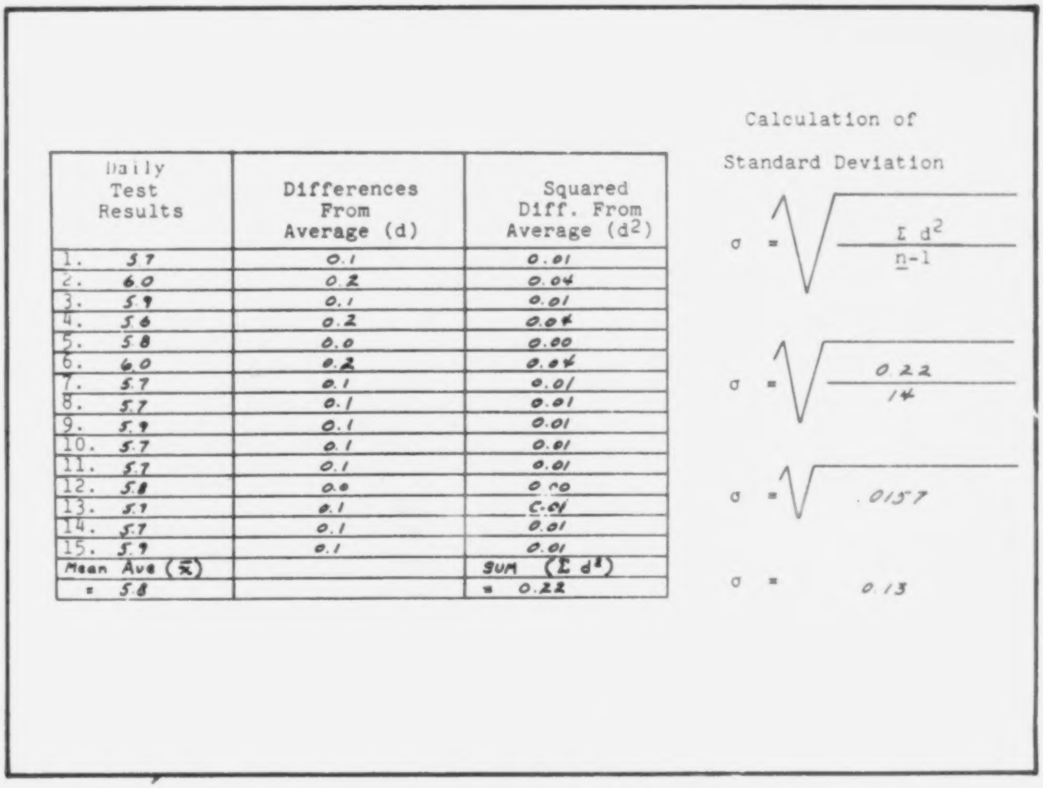
5. What does the symbol Σd^2 from the standard deviation formula mean?

6. Assuming random distribution and a statistically adequate number of cases, what percent of the values will fall within 1 σ below the mean?

085. Calculate the standard deviation and solve problems relating to the analysis of quality control data.

Daily Records. The calculation of standard deviation is based on a record of daily results which cover a period of not less than 15 days, and in most cases at least 21 days. A chart, as shown in figure 8-4, is very helpful. For purposes of illustration, the values for a series of potassium determinations have been entered in figure 8-4. The sum of these values divided by the number of tests gives the average value. Note that the difference of each daily result from the average is entered in column 2. The difference is then squared, as shown in column 3 of this figure. The sum of the differences is entered in your data table and is applied in the standard deviation formula as shown. To complete the formula, the number of daily results used, −1, is determined and is inserted in the formula as "n −1." The division quotient is calculated, and finally the square root of the quotient is obtained.

Once the standard deviation is established, you can begin to control the quality of your work. Assume that the mg/dl values for daily glucose procedures are charted and the standard deviation is calculated as 3 mg/dl. These values may be placed on a graph, as indicated in figure 8-5. As each daily result (numbered consecutively) is entered on the graph, you will immediately know whether you are within the established confidence limits. For the range of values plotted in figure 8-5, one standard deviation unit represents 3 mg/dl. It is important to realize that translation of SD units to mg/dl depends upon the range of values used to establish the curve. For example, in figure 8-5 ± 2 SD units would represent an allowable range of ± 6 mg/dl, and that would be the allowable range for the control serum to be in control at a concentration of 100 mg/dl. At a level of 300 mg/dl the range of allowable error would be ± 18 mg/dl, not ± 6 mg/dl, implying that if ± 2 SD units represent ± 6 mg/dl for the allowable range of the serum control at 100 mg/dl, then at a level of 300 mg/dl the allowable range would be ± 18 mg/dl.



Daily Test Results	Differences From Average (d)	Squared Diff. From Average (d^2)
1. 5.7	0.1	0.01
2. 6.0	0.2	0.04
3. 5.9	0.1	0.01
4. 5.6	0.2	0.04
5. 5.8	0.0	0.00
6. 6.0	0.2	0.04
7. 5.7	0.1	0.01
8. 5.7	0.1	0.01
9. 5.9	0.1	0.01
10. 5.7	0.1	0.01
11. 5.7	0.1	0.01
12. 5.8	0.0	0.00
13. 5.9	0.1	0.01
14. 5.7	0.1	0.01
15. 5.9	0.1	0.01
Mean Ave ($\bar{x}$)		SUM (Σd^2)
= 5.8		= 0.22

Calculation of

Standard Deviation

$$\sigma = \sqrt{\frac{\Sigma d^2}{\underline{n}-1}}$$

$$\sigma = \sqrt{\frac{0.22}{14}}$$

$$\sigma = \sqrt{.0157}$$

$$\sigma = 0.13$$

Figure 8–4. Data chart for entering test results and expressing standard deviation (values for potassium are entered).

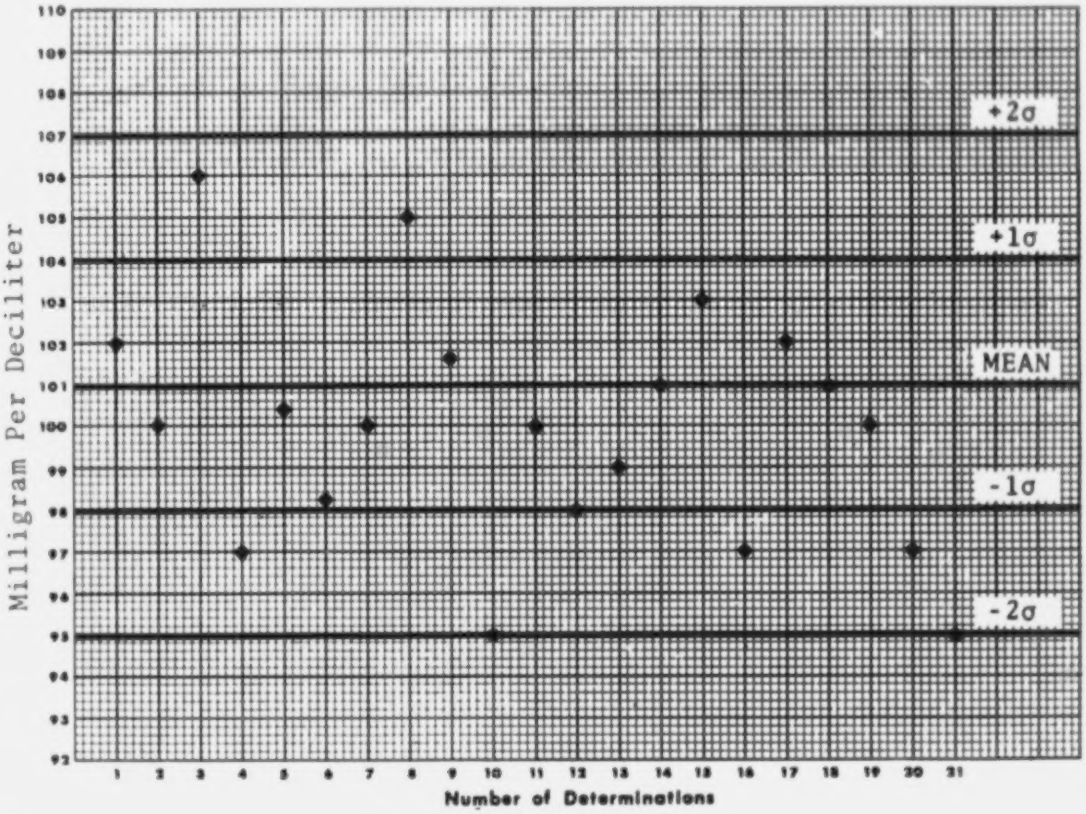


Figure 8-5. Quality control chart for glucose.

Certain steps are necessary in analyzing the data you have recorded. If you are outside confidence limits, record your results and repeat the entire set of analyses, including another aliquot of the control. If the repeat results are in control, they may be reported; if not, the procedure, reagents, instrument, and your own performance should be checked for error. It must be emphasized that repeating out-of-control runs are essential operations. Data is best analyzed as it becomes available.

Plotting the daily control results helps to obtain a good picture of the whole quality control program, including the day-to-day variation.

Final Advice on Human Error. There is a natural tendency, "human bias," to take readings or round off calculations to favor the expected results. This often happens when a test is repeated or a control sample with known value is analyzed. You should make a conscious effort to avoid this type of bias.

You should, of course, make every effort to avoid mistakes, but when mistakes do occur, they should be reported to a supervisor for appropriate action. One of the outstanding qualities of a good supervisor is to recognize honest technicians as an essential part of the quality control program, and he or she will encourage their cooperation to insure the accuracy of all reported data. Since questionable results are potentially more harmful to the patient than no results, complete honesty by the entire laboratory staff is very important.

Exercises (085):

1. You have determined a series replicate analyses on a control serum for glucose. Calculate the average (mean) value for the series, using the following values:

Specimen	*mg/dl Glucose*
1	118
2	115
3	111
4	114
5	117
6	117
7	120
8	116
9	112
10	117
11	122
12	119
13	117
14	117
15	121
16	121
17	121
18	117
19	111
20	115
21	119

2. Calculate one standard deviation, using the information in exercise 1. The square root required may be found in table 8-1 and worksheet furnished in figure 8-6.

TABLE 8-1
TABLE OF SQUARE ROOTS

	SD		SD
$\sqrt{9.610}$	3.10	$\sqrt{10.24}$	3.20
9.672	3.11	10.30	3.21
9.734	3.12	10.37	3.22
9.797	3.13	10.43	3.23
9.860	3.14	10.50	3.24
9.923	3.15	10.56	3.25
9.986	3.16	10.63	3.26
10.050	3.17	10.69	3.27
10.110	3.18	10.76	3.28
10.180	3.19	10.82	3.29

Test Results	Differences From Average (d)	Squared Diff. From Average (d^2)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
18.		
19.		
20.		
21.		
Mean Ave ($\bar{x}$) =		Sum (Σd^2) =

Calculation of Standard Deviation

$$\sigma = \sqrt{\frac{\Sigma d^2}{n - 1}}$$

$$\sigma = \sqrt{\frac{\qquad}{\qquad}}$$

$$\sigma = \sqrt{\qquad}$$

$$\sigma =$$

Figure 8-6. Standard deviation worksheet (objective 085, exercise 2).

3. What are the confidence limits for this glucose procedure, assuming an allowable error of 2 standard deviations?

4. Plot the values of the tests given in exercise 1 on graph paper furnished in figure 8-7. Indicate the mean value and the confidence limits of the test by drawing horizontal lines at these levels of concentration.

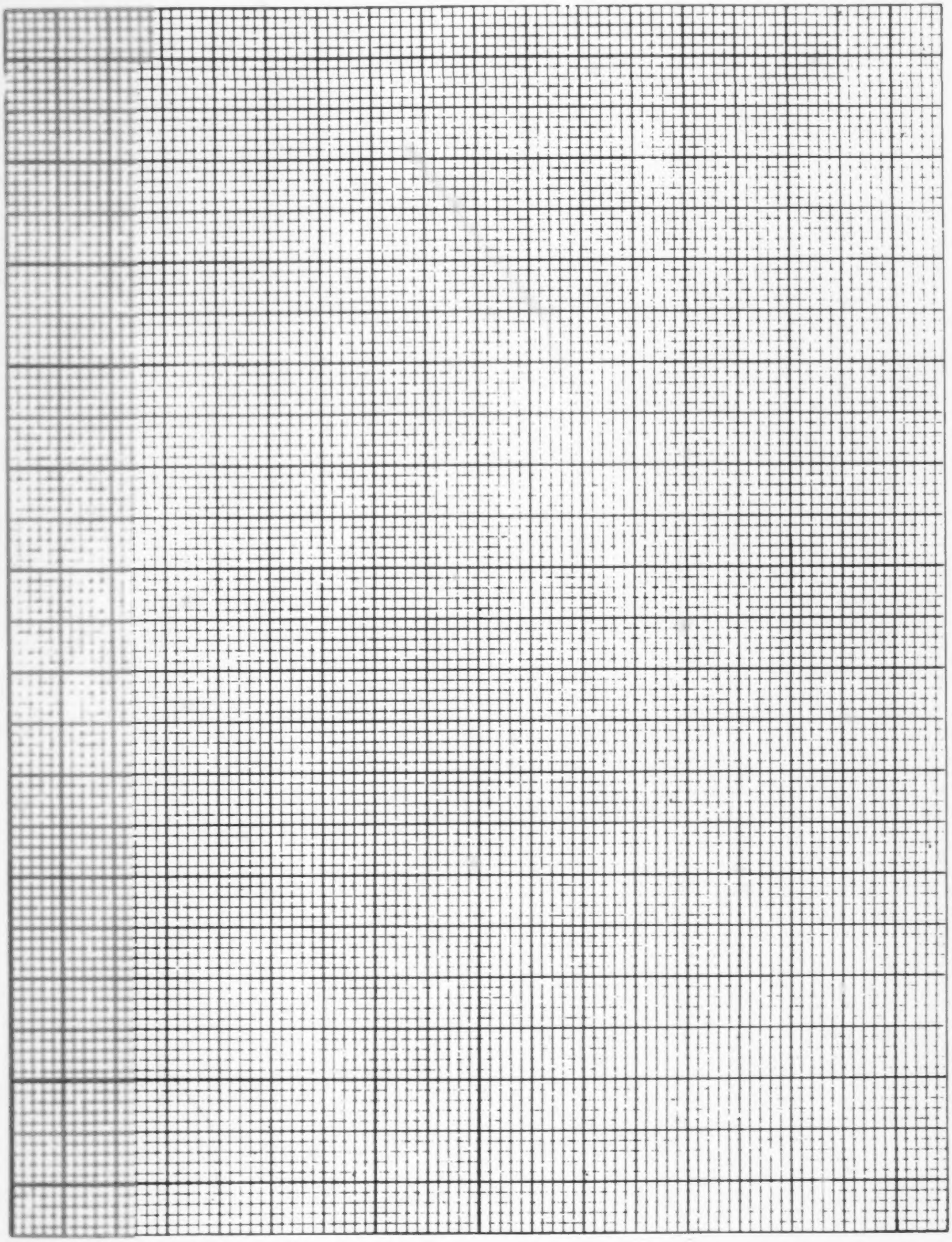


Figure 8-7. Graph paper (objective 085, exercise 4).

5. In the analysis of quality control data, should figures that are obviously out of range be recorded or discarded?

6. In 20 consecutive determinations, the sum of the squared difference is 159. What is the standard deviation?

086. Cite the significance of the USAF Graduate Evaluation Program in terms of how you may directly participate to affect the quality of your CDC and improve training in your specialty.

Field Evaluations. Field evaluations are a major aid in the quality control of formal resident courses, nonresident courses, CDCs, and computer directed training system (CDTS) courses and provide a source of information to determine the following factors:

a. Ability of recent graduates to perform their assigned job tasks at the level of proficiency, specified Air Force specialty training standards (STSs), course training standards (CTSs), or similar course control documents.

b. Extent to which acquired skills are used by recent graduates.

c. Extent to which graduates retain the knowledge and skill proficiency they have acquired.

d. Need to revise STS, CTS, CDC, or CDTS course to improve training.

e. Need for further evaluation of the education and training problem areas identified by field evaluations.

Thus, the major purpose is to provide feedback data to validate the effectiveness of your training. Let's examine the methods in the evaluation of graduates.

AF Form 1284, Training Quality Report. AF Form 1284 is used to provide feedback to ATC and other commands and organizations conducting education and training courses. It is used to document many types of training including resident courses, CDTS courses, and courses conducted by Phase II medical training facilities. When a person completing AF Form 1284 directly asks a graduate for personal information, the graduate must be given a Privacy Act Statement. The graduate, or anyone directly responsible for his or her training, may complete AF Form 1284. If you require additional assistance to complete the form, see your local representative from the OJT advisory service.

ATC Education and Training Programs. Further additional evaluation of training is accomplished by ATC through field evaluation visits, direct correspondence questionnaires, and evaluation of CDCs. Let's look at what is accomplished through field evaluation visits.

Field evaluation visits. Personnel from education and training activities, such as the School of Health Care Sciences (SHCS), are required to plan field evaluation visits to facilities that will provide evaluation of graduates who have been out of training for 4 to 6 months. Information is obtained from the graduate, the immediate supervisor, and others having knowledge of the graduate's performance. The specialty training standard (and other course documentations) are used as references in evaluating the graduates' frequency of use and ability to perform the tasks for which they are trained.

Direct correspondence questionnaire. Direct correspondence questionnaires pertain to graduate qualifications in terms of the knowledge and skill proficiency level reflected in the approved training standards or other course documentation. These questionnaires are sent to recent graduates and their supervisors. They are usually mailed by education and training activities (e.g., SHCS) to graduate receiving activities within 4 to 6 months after graduation. You should insure the timely and accurate completion of these questionnaires as with AF Form 1284.

CDC evaluation. The Extension Course Institute (ECI) has included specific questions in the last volume exercise booklet to solicit student opinion about the effectiveness. ECI gathers this information, summarizes the data, and provides the information to the training activity responsible for the CDC preparation. ECI also provides the training activity with statistics on each question from the volume review exercises and course examinations. These evaluation tools are used to point out problems with the CDC content in order that a followup evaluation can be made to determine corrective actions. As mentioned in the preface of this CDC, you may also write or call, using the AUTOVON, any inquiries about the CDC directly to the course author. Further, you may submit any recommendation in terms of improvement in techniques or on current methodologies whether during your enrollment or after your CDC is completed.

As you proceed through this course, you should assume that you will be requested to complete an evaluation questionnaire. if you feel that something should be changed, keep notes as you go through the course. Be honest in your evaluation, but be specific. If you feel that something is wrong, say so and, if possible, provide a recommended solution. Remember, this course is written for you; if you have an idea, let's hear from you.

Exercises (086):

1. Briefly explain the purpose of the USAF Graduate Evaluation Program.

2. What two general methods are used in the evaluation of graduates?

3. Who provides personnel from education and training activities with information they require while on field evaluation visits?

4. To whom are direct correspondence questionnaires sent?

5. What two ECI evaluation tools are used to improve the quality of your CDC?

6. How can you communicate directly with the course author concerning any recommendations that you may have to improve techniques or methodologies in this course?

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APPENDIX

Appendix A. Medical Laboratory Specialist
Appendix B. Medical Laboratory Technician
Appendix C. Medical Laboratory Superintendent
Appendix D. Histopathology Specialist
Appendix E. Histopathology Technician
Appendix F. Section 1. Tables of Elements Common to the Clinical Laboratory
Section 2. Periodic Chart
Section 3. Electron Configuration of Periods 1, 2, and 3 From the Periodic Chart

Appendix G. Summary of General Formulae
Appendix H. Mathematics

APPENDIX A

AFSC 92450
Semiskilled AFSC 92430
Helper AFSC 92410

AIRMAN AIR FORCE SPECIALTY

MEDICAL LABORATORY SPECIALIST

1. SPECIALTY SUMMARY

Tests and analyzes specimens of human origin and other substances by established scientific laboratory techniques to aid in diagnosis, treatment, and prevention of diseases or in support of medical research. *Related DOD Occupational Subgroup:* 311.

2. DUTIES AND RESPONSIBILITIES

a. *Performs hematological tests.* Accomplishes standardized quantitative and qualitative evaluation of erythrocytes, leukocytes, and thrombocytes. Examines stained blood smears microscopically and refers any abnormal cells to superiors. Performs coagulation studies on human blood and plasma. Assists in preparation of reagents needed in hematology.

b. *Performs urinalyses.* Performs chemical analyses and macroscopic and microscopic examinations of urine specimens. Prepares reagents for use in urinalysis.

c. *Performs chemical analyses.* Analyzes human material or other products submitted to laboratory, using photometric, colorimetric, titrimetric, radioisotope, or any other chemical or physical procedures applicable to clinical chemistry. Calibrates instruments employed in performance of foregoing analyses. Makes necessary calculations and reports data to superiors.

d. *Assists in blood bank duties.* Draws and processes blood aseptically by standardized techniques. Completely types blood of donors and recipients; assists in crossmatching of blood to establish donor-recipient compatibility, reporting any abnormal reactions to immediate supervisor. Assists in preparation of blood derivatives.

e. *Performs microbiological and serological tests.* Conducts procedures necessary to isolate and identify bacteria by gross and microscopic examination, staining, biochemical and immunological procedures, or any other determination of growth characteristics. Performs sensitivity tests on pathogenic bacteria. Assists in identification of viruses and fungi. Applies parasitological techniques to recover and identify parasites. Applies standard serological tests for identification of antibodies specific to diseases.

f. *Accomplishes general medical laboratory duties.* Performs microscopic and chemical examination of spinal fluid and gastric fluids. Conducts bacteriological and chemical examinations of food products, water, dairy products, and sewage incidental to preventive and veterinary medicine programs. Takes all necessary precautions to maintain safe conditions in laboratory for both laboratory and hospital personnel. Performs preventive maintenance procedures on laboratory equipment. Performs laboratory quality control procedures.

g. *Supervises medical laboratory personnel.* Schedules and assigns work to subordinates according to their ability and training. Evaluates methods employed and results of subordinates' work. Instructs and conducts on-the-job training in various techniques used in medical laboratory activities.

3. SPECIALTY QUALIFICATIONS

a. *Knowledge:*

(1) Knowledge of fundamentals of clinical chemistry; hematology; microbiology; blood banking; immunology; medical terminology; medical ethics; and principles of medical administration is mandatory. Possession of mandatory knowledge will be determined according to AFR 35-1.

(2) Knowledge of routine equipment maintenance is desirable.

b. *Education:*

(1) Completion of high school courses in algebra and chemistry is mandatory.

(2) Completion of high school courses in biology, zoology, and other basic sciences is desirable.

c. *Experience.* Experience in performing functions in urinalysis, hematology, bacteriology, serology, and chemistry is mandatory.

d. *Training.* Completion of a basic medical laboratory course is desirable.

e. *Other:* Normal color vision as defined by AFR 160-43 is mandatory.

APPENDIX B

AFR 39-1 Attachment 80 1 January 1982

A80-115/116
AFSC 92470

AIRMAN AIR FORCE SPECIALTY

MEDICAL LABORATORY TECHNICIAN

1. SPECIALTY SUMMARY

Performs established scientific laboratory techniques to aid in diagnosis, treatment, and prevention of diseases or in support of medical research; and supervises medical laboratory activities. *Related DOD Occupational Subgroup:* 311.

2. DUTIES AND RESPONSIBILITIES

a. *Performs chemical analyses.* Performs chemical analyses on all human material or products submitted to laboratory, using photometric, colorimetric, titrimetric, radioisotope, or any other chemical or physical procedures applicable to clinical chemistry. Calibrates and maintains all instruments. Sets up related curves or graphs for all instruments. Makes standard stock reagents and any special reagents needed. Reviews all chemistry procedures, assuring laboratory is using current or revised procedures.

b. *Performs hematological tests.* Accomplishes standardized quantitative and qualitative evaluation of erythrocytes, leukocytes, and thrombocytes. Examines stained blood and bone marrow smears microscopically for morphological features and reports any abnormalities or refers to superiors in charge for further evaluation. Makes all reagents needed.

c. *Performs microbiological tests.* Conducts procedures to isolate and identify bacteria, fungi, or viruses by gross and microscopic examination, staining, biochemical, and immunological procedures. Performs sensitivity tests on pathogenic organisms. Applies parasitological techniques to recover and identify parasites.

d. *Performs blood bank duties.* Accomplishes all techniques required for blood transfusion service. Draws and processes blood aseptically. Completely types blood of donors and recipients. Crossmatches blood to assure donor-recipient compatibility.

e. *Performs highly technical tasks related to medical technology.* Assists in research assignments in broad field of pathology. Prepares tissue for electron microscopy. Performs toxicology procedures incident to aerospace pathology and forensic medicine programs. Assists in epidemiological investigations.

f. *Supervises medical laboratory personnel.* Plans and schedules work. Establishes work methods and procedures. Determines supply requirements. Assigns duties to subordinates. Evaluates duty performance. Orients newly assigned personnel. Plans and conducts on-the-job training. Directs continuous organized training programs. Advises superiors of status of training and overall capabilities and efficiency of personnel. Supervises preparation of reports and maintenance of records.

g. *Reviews and updates instructions on all laboratory procedures.* Performs and monitors laboratory quality control procedures, taking necessary action to assure appropriate precision and accuracy.

3. SPECIALTY QUALIFICATIONS

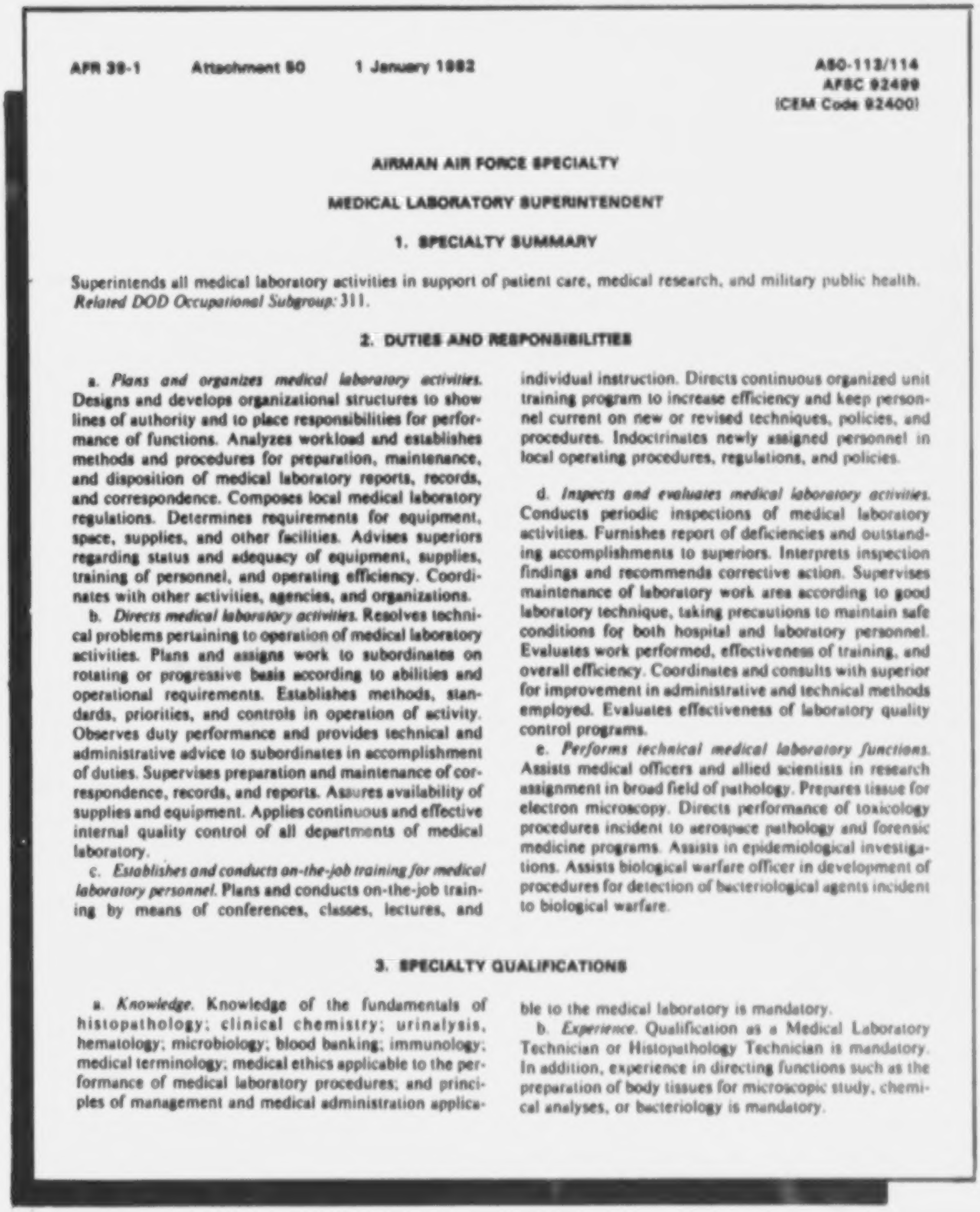
a. *Knowledge:*

(1) Knowledge of fundamentals of urinalysis; clinical chemistry; hematology; microbiology; blood banking; immunology; medical terminology; medical ethics applicable to performance of medical laboratory procedures; and principles of management and medical administration is mandatory. Possession of mandatory knowledge will be determined according to AFR 35-1.

(2) Knowledge of routine equipment maintenance is desirable.

b. *Experience.* Qualification as a Medical Laboratory Specialist is mandatory. In addition, experience in performing or supervising functions such as work normally performed in routine clinical laboratory (Class A Dispensary or Hospital), or specialty performance of tests or technical supervisory experience in clinical chemistry, bacteriology, toxicology, or virology at Class A, Class B, or analogous laboratory, or combination of foregoing types of experience, is mandatory.

c. *Training.* Completion of prescribed 7-level management course is mandatory.



AFR 39-1 Attachment 50 1 January 1982

A50-113/114
AFSC 92499
(CEM Code 92400)

AIRMAN AIR FORCE SPECIALTY

MEDICAL LABORATORY SUPERINTENDENT

1. SPECIALTY SUMMARY

Superintends all medical laboratory activities in support of patient care, medical research, and military public health. *Related DOD Occupational Subgroup:* 311.

2. DUTIES AND RESPONSIBILITIES

a. *Plans and organizes medical laboratory activities.* Designs and develops organizational structures to show lines of authority and to place responsibilities for performance of functions. Analyzes workload and establishes methods and procedures for preparation, maintenance, and disposition of medical laboratory reports, records, and correspondence. Composes local medical laboratory regulations. Determines requirements for equipment, space, supplies, and other facilities. Advises superiors regarding status and adequacy of equipment, supplies, training of personnel, and operating efficiency. Coordinates with other activities, agencies, and organizations.

b. *Directs medical laboratory activities.* Resolves technical problems pertaining to operation of medical laboratory activities. Plans and assigns work to subordinates on rotating or progressive basis according to abilities and operational requirements. Establishes methods, standards, priorities, and controls in operation of activity. Observes duty performance and provides technical and administrative advice to subordinates in accomplishment of duties. Supervises preparation and maintenance of correspondence, records, and reports. Assures availability of supplies and equipment. Applies continuous and effective internal quality control of all departments of medical laboratory.

c. *Establishes and conducts on-the-job training for medical laboratory personnel.* Plans and conducts on-the-job training by means of conferences, classes, lectures, and individual instruction. Directs continuous organized unit training program to increase efficiency and keep personnel current on new or revised techniques, policies, and procedures. Indoctrinates newly assigned personnel in local operating procedures, regulations, and policies.

d. *Inspects and evaluates medical laboratory activities.* Conducts periodic inspections of medical laboratory activities. Furnishes report of deficiencies and outstanding accomplishments to superiors. Interprets inspection findings and recommends corrective action. Supervises maintenance of laboratory work area according to good laboratory technique, taking precautions to maintain safe conditions for both hospital and laboratory personnel. Evaluates work performed, effectiveness of training, and overall efficiency. Coordinates and consults with superior for improvement in administrative and technical methods employed. Evaluates effectiveness of laboratory quality control programs.

e. *Performs technical medical laboratory functions.* Assists medical officers and allied scientists in research assignment in broad field of pathology. Prepares tissue for electron microscopy. Directs performance of toxicology procedures incident to aerospace pathology and forensic medicine programs. Assists in epidemiological investigations. Assists biological warfare officer in development of procedures for detection of bacteriological agents incident to biological warfare.

3. SPECIALTY QUALIFICATIONS

a. *Knowledge.* Knowledge of the fundamentals of histopathology; clinical chemistry; urinalysis, hematology; microbiology; blood banking; immunology; medical terminology; medical ethics applicable to the performance of medical laboratory procedures; and principles of management and medical administration applicable to the medical laboratory is mandatory.

b. *Experience.* Qualification as a Medical Laboratory Technician or Histopathology Technician is mandatory. In addition, experience in directing functions such as the preparation of body tissues for microscopic study, chemical analyses, or bacteriology is mandatory.

AFR 39-1 Attachment 80 1 January 1982

A80-121/122
AFSC 92481
Semiskilled AFSC 92431
Helper AFSC 92411

AIRMAN AIR FORCE SPECIALTY

HISTOPATHOLOGY SPECIALIST

1. SPECIALTY SUMMARY

Performs subprofessional duties at autopsies; prepares autopsy and surgical specimens of histopathology activities. *Related DOD Occupational Subgroup:* 311.

2. DUTIES AND RESPONSIBILITIES

a. *Performs subprofessional duties at autopsies.* Acts as an assistant at autopsies. Assists pathologist in opening abdominal, pleural, and cranial cavities, examining various organs, and procuring and handling specimens from these organs. Prepares remains for transfer to mortuary, to include cleaning and closing all incisions made by pathologist. Labels and stores autopsy specimens until pathologist makes final examination prior to fixing, embedding, and staining.

b. *Prepares autopsy and surgical tissue specimens.* Prepares specimens for fixation, dehydration, and empregnation processes by either manually or automatically sending specimens through series of formalins, alcohols, clearing agents, and paraffin. Embeds tissues and prepares paraffin blocks for cutting on rotary microtome. Attaches cut tissues on specially prepared microslides and clears paraffin from tissue. Runs tissue slides through staining processes. Labels stained slides by surgical or autopsy number. Submits finished tissue slides to pathologist along with pertinent pre-microscopic data received from originating agency and gross examination by pathologist. Performs special stains and procedures upon request of pathologist.

c. *Maintains histopathology records and instruments.* Maintains complete records of all autopsies and surgical specimens, to include orderly storing of paraffin blocks, filing of stained tissue slides by accession number, and so forth. Assembles and ships blocks, slides, and diagnostic reports to various military and civilian medical facilities. Maintains all autopsy and surgical instruments, to include sharpening of microtome blades and sharpening and cleaning of autopsy knives, scissors, and chisels.

d. *Supervises histopathology personnel.* Supervises subordinate personnel in proper use and care of instruments, slides, and records. Orients newly assigned personnel in operating policies and procedures. Plans and conducts on-the-job training. Advises superiors of status of training.

3. SPECIALTY QUALIFICATIONS

a. *Knowledge:*

(1) Knowledge of methods of fixing, staining, embedding, and cutting all types of tissue; properties of various biological stains and reagents; autopsy procedures; medical terminology; and medical ethics is mandatory. Possession of mandatory knowledge will be determined according to AFR 35-1.

(2) Knowledge of principles of management, medical administration, and routine equipment maintenance is desirable.

b. *Education:*

(1) Completion of high school courses in algebra and chemistry is mandatory.

(2) Completion of high school courses in biology, zoology, and other basic sciences is desirable.

c. *Experience.* Experience in specialty performance of tests or technical supervisory experience in histopathology at a Class A, Class B, or analogous laboratory is mandatory.

d. *Training:*

(1) Completion of a basic medical laboratory course is desirable.

(2) Completion of a histopathology course is desirable.

e. *Other:* A minimum of Grade 2 color vision as defined in AFM 160-17 is mandatory.

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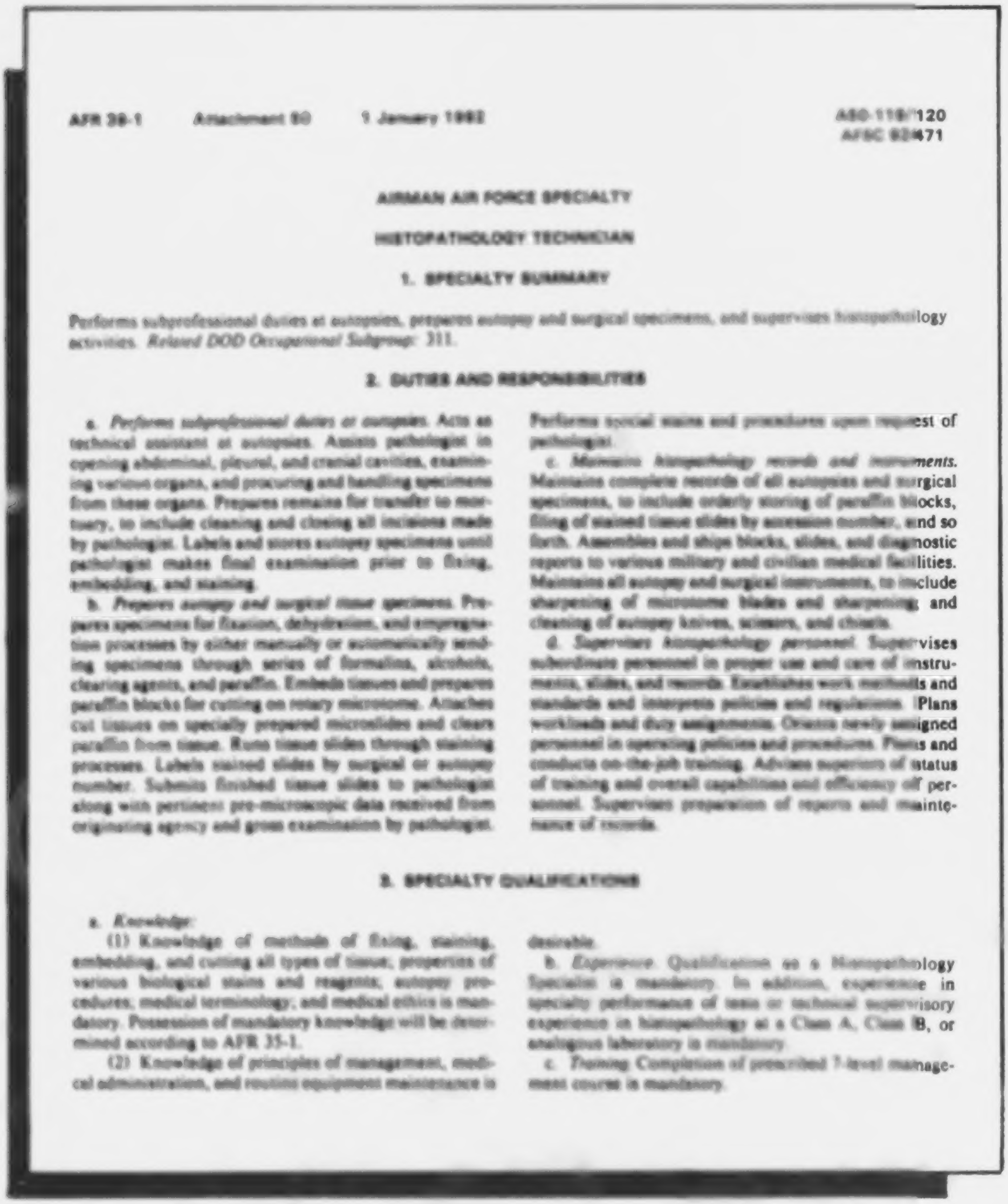


AFR 39-1 Attachment 80 1 January 1982

A80-119/120
AFSC 92471

AIRMAN AIR FORCE SPECIALTY

HISTOPATHOLOGY TECHNICIAN

1. SPECIALTY SUMMARY

Performs subprofessional duties at autopsies, prepares autopsy and surgical specimens, and supervises histopathology activities. *Related DOD Occupational Subgroup:* 311.

2. DUTIES AND RESPONSIBILITIES

a. *Performs subprofessional duties at autopsies.* Acts as technical assistant at autopsies. Assists pathologist in opening abdominal, pleural, and cranial cavities, examining various organs, and procuring and handling specimens from these organs. Prepares remains for transfer to mortuary, to include cleaning and closing all incisions made by pathologist. Labels and stores autopsy specimens until pathologist makes final examination prior to fixing, embedding, and staining.

b. *Prepares autopsy and surgical tissue specimens.* Prepares specimens for fixation, dehydration, and impregnation processes by either manually or automatically sending specimens through series of formalins, alcohols, clearing agents, and paraffin. Embeds tissues and prepares paraffin blocks for cutting on rotary microtome. Attaches cut tissues on specially prepared microslides and clears paraffin from tissue. Runs tissue slides through staining processes. Labels stained slides by surgical or autopsy number. Submits finished tissue slides to pathologist along with pertinent pre-microscopic data received from originating agency and gross examination by pathologist. Performs special stains and procedures upon request of pathologist.

c. *Maintains histopathology records and instruments.* Maintains complete records of all autopsies and surgical specimens, to include orderly storing of paraffin blocks, filing of stained tissue slides by accession number, and so forth. Assembles and ships blocks, slides, and diagnostic reports to various military and civilian medical facilities. Maintains all autopsy and surgical instruments, to include sharpening of microtome blades and sharpening and cleaning of autopsy knives, scissors, and chisels.

d. *Supervises histopathology personnel.* Supervises subordinate personnel in proper use and care of instruments, slides, and records. Establishes work methods and standards and interprets policies and regulations. Plans workloads and duty assignments. Orients newly assigned personnel in operating policies and procedures. Plans and conducts on-the-job training. Advises superiors of status of training and overall capabilities and efficiency of personnel. Supervises preparation of reports and maintenance of records.

3. SPECIALTY QUALIFICATIONS

a. *Knowledge:*

(1) Knowledge of methods of fixing, staining, embedding, and cutting all types of tissue; properties of various biological stains and reagents; autopsy procedures; medical terminology; and medical ethics is mandatory. Possession of mandatory knowledge will be determined according to AFR 35-1.

(2) Knowledge of principles of management, medical administration, and routine equipment maintenance is desirable.

b. *Experience.* Qualification as a Histopathology Specialist is mandatory. In addition, experience in specialty performance of tests or technical supervisory experience in histopathology at a Class A, Class B, or analogous laboratory is mandatory.

c. *Training.* Completion of prescribed 7-level management course is mandatory.

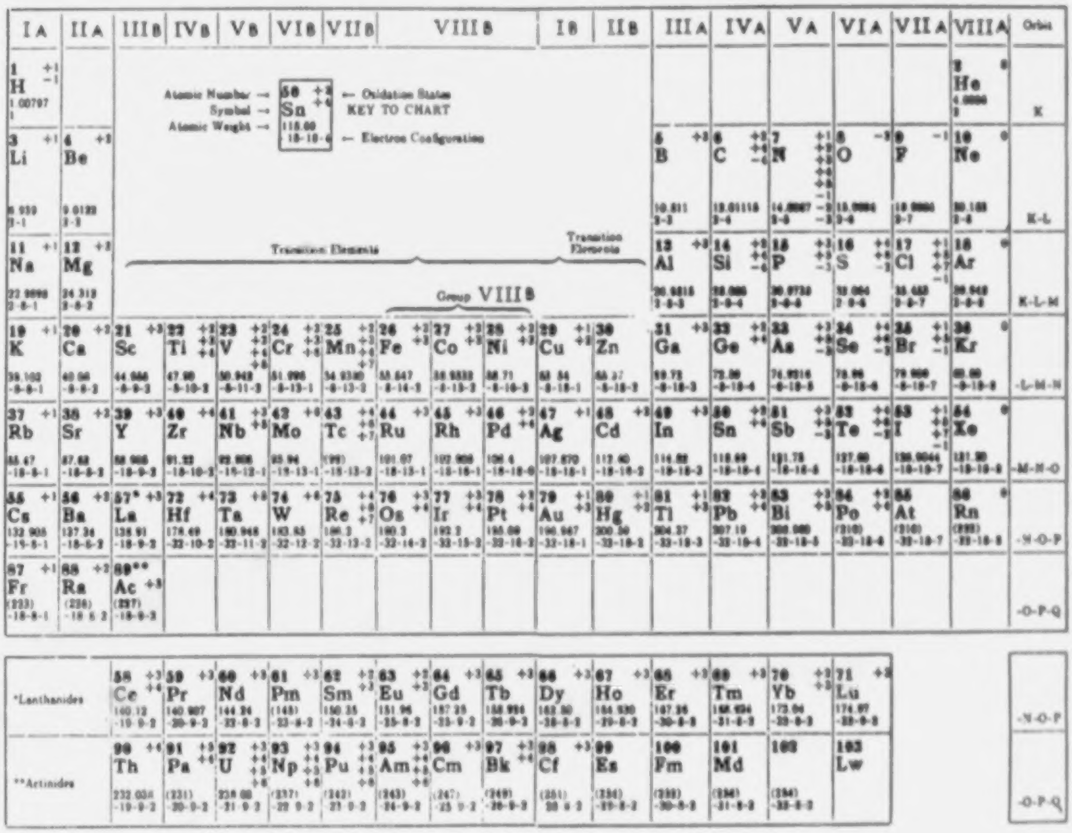
Section 1. *Table of Elements Common to the Clinical Laboratory*

This table provides a rapid means of referring to the atomic weight or atomic number of elements frequently encountered in the clinical laboratory.

NAME	*SYMBOL*	*ATOMIC NO.*	*ATOMIC WT.*
Aluminum	Al	13	26.98
Antimony	Sb	51	121.76
Arsenic	As	33	74.91
Barium	Ba	56	137.36
Bismuth	Bi	83	209.00
Bromine	Br	35	79.92
Calcium	Ca	20	40.08
Carbon	C	6	12.01
Chlorine	Cl	17	35.46
Chromium	Cr	24	52.01
Cobalt	Co	27	58.94
Copper	Cu	29	63.54
Fluorine	F	9	19.00
Gold	Au	79	197.00
Hydrogen	H	1	1.008
Iodine	I	53	126.91
Iron	Fe	26	55.85
Lead	Pb	82	207.21
Lithium	Li	3	6.94
Magnesium	Mg	12	24.32
Manganese	Mn	25	54.94
Mercury	Hg	80	200.61
Molybdenum	Mo	42	95.95
Nickel	Ni	28	58.71
Nitrogen	N	7	14.01
Osmium	Os	76	190.20
Oxygen	O	8	16.00
Palladium	Pd	46	106.4
Phosphorus	P	15	30.98
Potassium	K	19	39.10
Selenium	Se	34	78.96
Silicon	Si	14	28.09
Silver	Ag	47	107.88
Sodium	Na	11	22.99
Sulfur	S	16	32.07
Tin	Sn	50	118.70
Tungsten	W	74	183.86
Zinc	Zn	30	65.38

Section 2. *Periodic Chart* *

The periodic law states that chemical properties of elements are periodic functions of their atomic numbers. That is, if elements are arranged according to atomic number, they can be grouped in vertical columns in such a way that each element will have properties similar to the one directly above or below it. Following the Periodic Chart is a table showing the electron configuration of the elements of the first three periods.


Atomic Number →
Symbol →
Atomic Weight →
50 Sn 118.69 -18-18-4 +2 +4
← Oxidation States
KEY TO CHART
← Electron Configuration
Transition Elements
Transition Elements
Group VIII B

IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIIIB			IB	IIB	IIIA	IVA	VA	VIA	VIIA	VIIIA	Orbit
1 H																	2 He	K
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne	K-L
11 Na	12 Mg											13 Al	14 Si	15 P	16 S	17 Cl	18 Ar	K-L-M
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr	-L-M-N
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe	-M-N-O
55 Cs	56 Ba	57* La	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn	-N-O-P
87 Fr	88 Ra	89** Ac																-O-P-Q

*Lanthanides	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu	-N-O-P
**Actinides	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102	103 Lw	-O-P-Q

Numbers in parentheses are mass numbers of most stable isotope of that element.

---

* Adapted from Handbook of Chemistry and Physics, 47th Edition, The Chemical Rubber Company, Cleveland, Ohio. Values for atomic weights given in this table may differ slightly from the values listed in Section 1.

APPENDIX F (Cont'd)

104	105	106
+4		
(261)	(262)	(263)
32-10-2	-32-11-2	-32-12-2

PERIODIC TABLE OF THE ELEMENTS

APPENDIX F (Cont'd)

ELECTRON CONFIGURATION OF PERIODS 1, 2, and 3 FROM THE PERIODIC CHART

Atomic Number	Element	K	L	M	
1	Hydrogen	1			
2	Helium	2			Inert
3	Lithium	2	1		
4	Beryllium	2	2		
5	Boron	2	3		
6	Carbon	2	4		
7	Nitrogen	2	5		
8	Oxygen	2	6		
9	Fluorine	2	7		
10	Neon	2	8		Inert
11	Sodium	2	8	1	
12	Magnesium	2	8	2	
13	Aluminum	2	8	3	
14	Silicon	2	8	4	
15	Phosphorus	2	8	5	
16	Sulfur	2	8	6	
17	Chlorine	2	8	7	
18	Argon	2	8	8	Inert

APPENDIX G

Summary of General Formulae

This section provides a list of general formulae useful in clinical chemistry calculations.

---

1 PURPOSE: To determine the number of grams of solution required to prepare a W/V percent solution of a specific volume.

EQUATION: Grams of Solute Required Equals:

$$\frac{(\text{percent W/V}) \times (\text{volume in ml. of final solution})}{100}$$

---

2 PURPOSE: To determine the number of milliliters of solute required to prepare a V/V percent solution of a specific volume.

EQUATION: Milliliters of Solute Required Equals:

$$\frac{(\text{percent V/V}) \times (\text{volume in ml. of final solution})}{100}$$

159

---

3 **PURPOSE:** To determine the volume of a concentrated reagent which contains a desired weight of the pure compound in solution.

EQUATION: Milliliters of Solution Containing Required Weight Equals:

$$\frac{\text{g. of concentrated solution required}}{\text{specific gravity of concentrated solution}}$$

---

4 **PURPOSE:** To determine the number of milliliters of a concentrated W/W reagent required to prepare a W/V percent solution of a specific volume.

EQUATION: Milliliters Concentrated Reagent to be Used Equals:

$$\frac{\begin{matrix}\text{ml. equivalent} \\ \text{to 1 g. (list)}\end{matrix} \times \begin{matrix}\text{percent} \\ \text{W/V desired}\end{matrix} \times \begin{matrix}\text{desired final} \\ \text{volume in ml.}\end{matrix}}{100}$$

---

5 **PURPOSE:** To determine the weight of a compound required to prepare a solution of a specific molarity and volume.

EQUATION: Grams of Compound Required Equals:

$$\frac{\begin{matrix}\text{molecular} \\ \text{weight}\end{matrix} \times \begin{matrix}\text{desired} \\ \text{molarity}\end{matrix} \times \begin{matrix}\text{final volume} \\ \text{in ml.}\end{matrix}}{1000}$$

---

6 **PURPOSE:** To determine the number of mililiters of a concentrated W/W reagent required to prepare a solution of a specific molarity and volume.

EQUATION: Milliliters of Concentrated Reagent to be Used Equals:

$$\frac{\begin{matrix}\text{molecular} \\ \text{weight}\end{matrix} \times \begin{matrix}\text{desired} \\ \text{molarity}\end{matrix} \times \begin{matrix}\text{final volume} \\ \text{in ml.}\end{matrix} \times \begin{matrix}\text{factor} \\ \text{from list}\end{matrix}}{1000}$$

---

7 PURPOSE: To determine the weight of a compound required to prepare a solution of a specific normality and volume.

EQUATION: Grams of Compound Required Equals:

$$\frac{\text{molecular weight} \times \text{desired normality} \times \text{final volume in ml.}}{(\text{total positive valence}) \times 1000}$$

---

8 PURPOSE: To determine the number of milliliters of a concentrated W/W reagent required to prepare a solution of a specific normality and volume.

EQUATION: Milliliters Concentrated Reagent to be Used Equals:

$$\frac{\text{molecular weight} \times \text{desired normality} \times \text{final volume in ml.} \times \text{factor from list}}{(\text{total positive valence}) \times 1000}$$

---

9 PURPOSE: To determine the number of milliliters of a concentrated stock solution to be diluted to obtain a more dilute solution.

EQUATION: Milliliters Stock Concentrated Solution to be Diluted Equals:

$$\frac{\text{ml. dilute solution desired} \times \text{concentration of dilute solution}}{\text{concentration of stock solution}}$$

---

10 PURPOSE: To convert an electrolyte concentration from mg. per 100 ml. to mEq./L.

EQUATION. mEq./L. (Electrolyte) Equals:

$$\frac{(\text{mg. per 100 ml.}) \times (10)}{\text{milliequivalent weight}}$$

---

11 PURPOSE: To determine the exact normality of an acid or base following a titration.

EQUATION: Volume of Acid Used X Normality of Acid Equals:

$$(\text{volume of base required}) \times (\text{normality of base})$$

---

12 PURPOSE: To determine the actual concentration of standard used in visual colorimetric and spectrophotometric analyses.

EQUATION: C_s Equals:

$$\begin{array}{c}\text{amount of standard}\\ \text{in 1 ml. of solution}\end{array} \times \begin{array}{c}\text{no. ml. of this standard}\\ \text{solution used in the test}\end{array}$$

---

13 PURPOSE: To determine the actual volume of specimen used in a chemical analysis.

EQUATION: V Equals:

$$\frac{\begin{array}{c}\text{ml. of specimen used to}\\ \text{prepare PFF or ml. of}\\ \text{specimen initially diluted}\end{array} \times \begin{array}{c}\text{ml. of PFF or ml.}\\ \text{of diluted specimen}\\ \text{actually used in test}\end{array}}{\begin{array}{c}\text{total volume in which original specimen is diluted,}\\ \text{e.g., total volume of specimen and precipitating}\\ \text{agents when preparing PFF}\end{array}}$$

---

14 PURPOSE: To determine the concentration of an analyzed specimen when the spectrophotometer is used and the final volume of the standard and unknown are identical.

EQUATION: C_u Equals:

$$\frac{OD_u}{OD_s} \times C_s \times \frac{100}{V}$$

15 PURPOSE: To determine the concentration of an analyzed specimen when the spectrophotometer is used, and the final volumes of the standard and unknown are not the same.

EQUATION: C_u Equals:

$$\frac{OD_u}{OD_s} \times C_s \times \frac{100}{V} \times \frac{\text{final volume of unknown}}{\text{final volume of standard}}$$

This section provides a summary of general formulae which is useful in clinical chemistry calculations. The following list of reagent factors is required to solve formulae 4, 6, and 8. This factor is the volume of concentrated reagent containing 1 g. of pure reagent. It is equivalent to the reciprocal of the specific gravity of the reagent or $\frac{1}{\text{sp.gr.}}$.

Concentrated Reagent	*Number of ml. that will be approximately equivalent to 1 g. of pure solute*
Acetic acid, glacial......	0.96
Ammonium hydroxide........	3.97
Hydrochloric acid.........	2.30
Nitric acid...............	1.01
Phosphoric acid...........	0.70
Sulfuric acid.............	0.57

Equations

An equation is a mathematical expression of equalities. In clinical chemistry computations, it is often necessary to transpose members of an equation in order to solve for an unknown. This section explains some of the mechanisms involved in solving equations. Consider the following example of an equation.

$$\frac{OD_u}{OD_s} \text{ X } C_s \text{ X } \frac{100}{V} = C_u$$

Whatever mathematical operation is performed on the left side of the equation must be performed on the right side to avoid disturbing the equality. For example, each side could be multiplied by 100 and the expression would still hold true. If all the values except C_u are known, it involves no more than simple arithmetic to solve for C_u. But if all of the values except OD_s are known, it is necessary to get OD_s alone on one side of the equal sign. The way this is accomplished in algebra is to perform a mathematical procedure opposite to that expressed on the side of the equation in which the quantity appears, in this case OD_s. Note that in the original equation OD_s is divided out on the left side; hence multiplying both sides of the equation by OD_s will cause it to appear on the right side as follows.

$$OD_u \text{ X } C_s \text{ X } \frac{100}{V} = C_u \text{ X } OD_s$$

Now divide both sides by C_u, since a multiplication is expressed on the right side of the equation. The equation then becomes

$$\frac{OD_u \text{ X } C_s \text{ X } 100}{V \text{ X } C_u} = OD_s$$

and is said to be expressed in terms of OD_s. The reason for moving OD_s to the right side of the equation is to get it alone on one side with minimum effort. This could also have been accomplished by the following steps.

Step 1: Divide each side by C_s.

Step 2: Multiply both sides by $\frac{V}{100}$.

Step 3: Divide both sides by OD_u.

Step 4: Invert both sides, since OD_s now appears as $\frac{1}{OD_s}$ on the left side as a result of dividing out OD_u.

Note that regardless of how you solve for the unknown, a factor of the unknown is always eliminated by a mathematical procedure opposite to that expressed in the equation. Consider the equation $x + 20 = y$. In order to solve for x, subtract 20 from both sides, because subtraction is the opposite of the addition expressed on the left side of the equation. The result would be $x = y - 20$. It must be remembered that although multiplication and division can be accomplished in any order, one cannot multiply or divide across plus or minus signs. Consequently, expressions such as $y - 20$ should be placed in parentheses and written $(y - 20)$ if there are other factors of that expression. For example, the equation

$x = \frac{y - 20}{10}$ is best written $x = \frac{(y - 20)}{10}$ to emphasize this expression is a quantity and *not* equivalent to $\frac{(y - 2)}{1}$. It makes no difference in which factor of an equation multiplication or division is expressed. For example

$$\frac{OD_u}{OD_s} \times C_s \times \frac{100}{V}$$

is the same as

$$OD_u \times \frac{C_s}{V} \times \frac{100}{OD_s} \qquad \text{or} \qquad \frac{OD_u \times C_s \times 100}{OD_s \times V}$$

If V is equal to 100, the factor of 100 in the numerator would cancel out, which is not the case when the numerator involves addition $(OD_u + C_s + 100)$ rather than multiplication.

The fundamental operations of mathematics may be summarized in three laws. 1. *The commutative law* states that regardless of the order of addition or multiplication, the result is the same. Example: $x + y = y + x$. 2. *The associative law* states that the product of three or more factors and the sum of three or more terms is the same regardless of the manner in which they are grouped. Example: $x + (y + z) = (x + y) + z = x + y + z$ and $x(yz) = (xy)z = xyz$. 3. *Distributive law* states that two or more terms multiplied by a single factor is equal to the sum of the products of each term of the expression multiplied by the single factor. Example: $a(x+y-z) = ax + ay - az$.

Answers for Exercises

CHAPTER 1

Reference:
001 – 1. To provide timely and reliable laboratory support of value in the diagnosis, treatment, and prevention of diseases affecting the health and welfare of U.S. Air Force personnel and their dependents.
001 – 2. The size, the scope of treatment provided, and the number, initiative, and/or training of assigned laboratory personnel.
001 – 3. Class A, Epidemiology Division USAF SAM, or to Army or Navy laboratories.
001 – 4. Commercial laboratories, ''downtown,'' or community hospital laboratories, and state public health laboratories.
001 – 5. (1) c.
 (2) d.
 (3) b.
 (4) b.
 (5) a.
 (6) a.
 (7) d.

002 – 1. AFR 35–1, Military Personnel Classification Policy.
002 – 2. A combination of the Medical Career Field with the cytotechnology subdivision.
002 – 3. (1) c.
 (2) b.
 (3) a.
 (4) d.

003 – 1. High school courses in chemistry and algebra are mandatory, and courses in biology, zoology, and other basic sciences are desirable.
003 – 2. Must be evaluated by a selected corp of qualified instructors and supervisors of the apprentice medical laboratory specialist course and, if selected, are proficiency advanced through course 3ABR92430. They are awarded the AFSC 92430 upon completion of this course.

004 – 1. Must complete CDC 90411, 90412, and 90413, and 12 months OJT, and have supervisory and technical experience. Complete PME Phase II, USAF Supervisor's course or Phase III, Command NCO Leadership School.
004 – 2. AFR 39–1, *Airman Classification Regulation*.
004 – 3. Performs established scientific laboratory techniques to aid in diagnosis, treatment, and prevention of diseases or in support of medical research, and supervises medical laboratory activities.
004 – 4. None.
004 – 5. The AKT is designed and written at the semiskilled level; the SKT is written at the skilled and advanced levels and is designed for promotion purposes.
004 – 6. Basic course.
004 – 7. Lateral course.
004 – 8. An airman who is awarded a semiskilled level of an AFSC based on prior civilian or military service or schooling.

005 – 1. Prior civilian experience or military occupational experience or training.
005 – 2. Block tests and the practical examination.
005 – 3. 92430, Apprentice Medical Laboratory.
005 – 4. 92450, Medical Laboratory Specialist.
005 – 5. 6 months; the commander.
005 – 6. 22nd month.
005 – 7. Under the below-the-zone promotion.
005 – 8. 10 percent.
005 – 9. Staff sergeant.

006 – 1. When he or she receives promotion to staff sergeant.
006 – 2. 1 year.
006 – 3. Ask your supervisor to request an extension from ECI before the year is completed.
006 – 4. 1 year.
006 – 5. PME Phase II, USAF Supervisor's course, or PME Phase III, Command NCO Leadership School.
006 – 6. The board will recommend an extension of time to complete the training.
006 – 7. Master sergeant.

007 – 1. F. Has a minimum of 12 months in grade, possesses a 7 level, is recommended by his or her supervisor, and has completed the USAF Senior NCO Academy Associate Program.
007 – 2. F. Selection is made by promotion boards meeting annually at Headquarters Air Force Military Personnel Center, Randolph AFB TX.
007 – 3. T.
007 – 4. T.
007 – 5. T.

008 – 1. F. Chief master sergeant.
008 – 2. F. Medical laboratory and cytotechnology superintendents.
008 – 3. T.

009 – 1. (1) Fair and equal consideration for promotion; (2) visibility; and (3) objectivity.
009 – 2. Two points for every year in service (6 months or less, 1 point; more than 6 months, 2 points).
009 – 3. 460.
009 – 4. Add all APR scores (0 to 9) of the last 5 years not to exceed 10 APRs. Divide the sum by the number of APRs used. Multiply by 15.
009 – 5. Three points.
009 – 6. 165.
009 – 7. Master sergeant, technical sergeant, and staff sergeant.

010 – 1. (1) c.
 (2) a, b.
 (3) b, c.
 (4) a, b, c.
 (5) c.
 (6) b, c.
 (7) b, c.
 (8) a.
 (9) a.
 (10) a.
 (11) b, c.
 (12) b, c.

CHAPTER 2

011 – 1. (1) a.
 (2) b.
 (3) d.
 (4) f.
 (5) c.
 (6) a, b.
 (7) f.
 (8) d.
 (9) d.
 (10) e.
 (11) a.

012 – 1. (1) f.
 (2) c.
 (3) d.
 (4) e.
 (5) b.
 (6) g.
 (7) h.
 (8) a.
 (9) j.
 (10) k.
 (11) m.
 (12) n.
 (13) l.
 (14) i.

013 – 1. F. Every item you order must appear on the issue list even if it is back ordered.
013 – 2. T.
013 – 3. T.
013 – 4. F. Normally, justification is not required for items less than $300 ordered on DD Form 1348–6.
013 – 5. T.
013 – 6. F. Nonstandard items are those NOT found in Federal supply catalogs.
013 – 7. F. Nonstandard replacement parts, over $300 for nonstandard equipment, are ordered on AF Form 601, Equipment Action Request.
013 – 8. F. They may be turned into Medical Equipment Management Office (MEMO).
013 – 9. T.
013 – 10. F. You should also mention the condition code as determined by the medical equipment repair technician.
013 – 11. T.
013 – 12. T.
013 – 13. F. The Using Activity Back Order Report reflects the current status of equipment due-ins/due-outs for both supplies and equipment.
013 – 14. T.

014 – 1. (1) To insure that the items are available when needed; and (2) to prevent accumulation of excess supplies.
014 – 2. 2 weeks.
014 – 3. Your order rate should correspond to your consumption rate.
014 – 4. Medical Supply may end up overstocked or without the item altogether.
014 – 5. Advise Medical Supply personnel of the change.

015 – 1. T.
015 – 2. T.
015 – 3. F. You should also consider any upcoming changes in the workload and types of examinations that might affect your requirements.
015 – 4. T.
015 – 5. T.

016 – 1. The obligation to care for Air Force property with which you are associated.
016 – 2. Proper care.
016 – 3. The obligation to pay for an item if it is lost, damaged, or destroyed as a result of maladministration or negligence.
016 – 4. Fill out DD Form 113, Cash Collection Voucher, and pay cash, or complete DD Form 362, Statement of Charges for Government Property, Lost, Damaged, or Destroyed, authorizing the Government to deduct the amount from your paycheck.
016 – 5. When an individual does not admit liability or when the amount is $500 or more.
016 – 6. Makes a detailed and impartial investigation of the circumstances connected with the loss, damage, or destruction of the property.

017 – 1. To protect all Air Force personnel from work-related deaths, injuries, and occupational illness.
017 – 2. That they provide Air Force personnel a safe and healthful environment in which recognized hazards have been eliminated or controlled.
017 – 3. Compliance with safety, fire, and health guidance.
017 – 4. Engineering changes, administrative controls, or revised procedures.
017 – 5. Supervisors, safety officers, or the bioenvironmental engineers.

018 – 1. To assist managers of USAF medical organizations in maintaining a safe environment and to administer a safety program that conforms to Air Force directives.
018 – 2. You are a highly trained and valuable resource.
018 – 3. You and your fellow workers.
018 – 4. (1) Be aware of safety hazards; (2) follow policies and procedures designed for your protection; and (3) report all incidents or accidents so that steps may be taken to prevent recurrence.

019 – 1. (1) a.
(2) a, b, e, l.
(3) d.
(4) g.
(5) j.
(6) g.
(7) f.
(8) b.
(9) i.
(10) c.
(11) h.
(12) k.
(13) k.
(14) g.

020 – 1. Before leaving the laboratory, before and between patient contact, and before or after smoking.
020 – 2. They are labeled as to their hazardous qualities and kept in an explosion-proof refrigerator.
020 – 3. 10 gallons or a week's supply.
020 – 4. In a space with good ventilation, preferably in a fume hood.
020 – 5. If the cylinder falls it can cause the outlet valve to rupture, and the cylinder may act like a torpedo and inflict serious injury.
020 – 6. Since propane is heavier than air, a little leaking gas can flow along the laboratory counter top to be ignited by the burning cigarette.
020 – 7. Sodium azide poured into drains reacts with metals in the plumbing and forms a powerful contact-sensitive explosive.
020 – 8. 5 percent sodium hypochlorite.
020 – 9. Always add acid to water (Remember AWA!).

021 – 1. Wash injured eye thoroughly with plain water for 15 minutes without delay.
021 – 2. They prevent thorough irrigation and must be removed to prevent further injury by chemicals.
021 – 3. Wash away the chemical completely, as quickly as possible, with copious quantity of running water until all traces of the chemical have been removed.
021 – 4. A mild alkaline solution of sodium bicarbonate.
021 – 5. 5 percent ammonium chloride or 5 percent zinc chloride.
021 – 6. None, nor anything without the sanction of the attending physician.

022 – 1. It can point to accident trends, high hazard areas, and frequency and severity of incidents involving personnel and equipment; protection against false claims by unscrupulous individuals.
022 – 2. The diagnosis, treatment, and prognosis is made easier; analysis of health records for future disability claims is easier to interpret and evaluate.
022 – 3. AF Form 765, Hospital Incident Statement.
022 – 4. AF Form 765, Hospital Incident Statement, and AF Form 457, USAF Hazard Report.

CHAPTER 3

023 – 1. It is compiled by the laboratory officer, superintendent or NCOIC, or pathologist and coordinated with the other hospital services.
023 – 2. All specific examinations and services (routine and emergency) within the capability of the laboratory.
023 – 3. First copy for the patient's record, the second copy to the doctor, and the third copy in the lab.
023 – 4. Computerized laboratories may file a composite daily master computer log.
023 – 5. Alphabetically by the patient's last name and test category.
023 – 6. 12 months.

024 – 1. Departmental and field publications.
024 – 2. Field publications.
024 – 3. It provides an alphabetical listing of the basic subject series and a numerical listing of each publication subject and title.
024 – 4. Series No. 161.
024 – 5. Field publication.
024 – 6. Series No. 169.

025 – 1. The office of Personnel and Administrative Services; publication number, title, justification and the number of publications required.
025 – 2. Publication Distribution Office.
025 – 3. Medical Materiel or the Medical Library.

026 – 1. AFR 12–20, *Management of Documentation.*
026 – 2. AFR 12–50, *Disposition of Air Force Documentation.*
026 – 3. Subjective.
026 – 4. T10–1, R2, R3.
026 – 5. AF Form 80, Files Maintenance and Disposition Plan.
026 – 6. AF Form 82, Files Disposition Control Label.

027 – 1. Show an interest in them by discussing their backgrounds and interests, and find out if they have any problems and provide them with assistance.
027 – 2. Assign them to a position quickly.
027 – 3. Introduce them to the physical layout and personnel in the organization. Show them such places as where they eat lunch and show them where they may set their coats, hats, or secure their personal belongings. Also, explain the local policies as regards duty hours, lunch periods, breaks, etc.
027 – 4. Instruct them on their new job, or provide a qualified instructor. Check their progress and encourage questions, make corrections, and give them encouragement for positive efforts.

028 – 1. There is never any justification for reporting information outside the "need-to-know" group.
028 – 2. A lawsuit against the Air Force.
028 – 3. The NCOIC's physician.
028 – 4. The patient's supervisor.
028 – 5. Let them know and feel that you trust them and their work.
028 – 6. Correct them in private.

029 – 1. Don't ignore your patients. Take the time to inform them as to the reason for the delay. They will be inclined to be more understanding when they know what is going on.
029 – 2. The specimen was misnumbered or mislabeled.
029 – 3. Take a few seconds to doublecheck your results and report all discrepancies.
029 – 4. Process the work quickly and accurately; then when the workload slows down, talk with the physician about the temporary problem. Listen to his or her side. The physician should be willing to hear your side and act accordingly to help reduce the workload whenever possible.
029 – 5. Knowing your job and how to do it.
029 – 6. Competency.

030 – 1. The reputation of every member of the medical facility.
030 – 2. The patient's needs.

031 – 1. First, try to take a message and have the technician return the call. If this doesn't satisfy the caller, take a short message and hand it to the technician. A small hint: If the message really isn't that important, or if the caller won't give you the message, the "hold" button may be quite convenient.
031 – 2. Refer the patient to the doctor for advice.
031 – 3. a. 3.
b. 2.
c. 4.
d. 1.

032 – 1. Budgeting and financial planning, manpower and staffing, facilities and equipment procurement, operational capability, analysis and patient management.
032 – 2. Monthly.
032 – 3. It is transmitted to major command and subsequently to Headquarters USAF.
032 – 4. Medical resource management office.
032 – 5. AFM 168–695, *Medical Administrative Management System — Base.*
032 – 6. Inpatients and outpatients.
032 – 7. Histopathology, those tests and procedures for other hospitals and clinics, or for base medical services not engaged in the treatment or examination of patients.

033 – 1. Clinical laboratory workload and personnel data.
033 – 2. The number of procedures to include standards and quality control procedures, specimen procurement (collection), specimen dispatch, and clerical functions in the procedure totals.
033 – 3. They are not counted as separate procedures.
033 – 4. The CAP — *Laboratory Workload Reporting Method.*
033 – 5. The Air Force Consultant in Pathology.
033 – 6. A test, or procedures done to solve unforeseen problems encountered in a patient sample run.
033 – 7. No. They do not qualify as repeat tests because they were not done to solve unforeseen problems encountered in a patient sample run.
033 – 8. The Air Force Consultant in Pathology.
033 – 9. Standards, controls, repeats, clerical functions, specimen procurement, and specimen dispatched.
033 – 10. Line 18.
033 – 11. A surgical specimen shall be equal to one complete surgical pathology report having a single surgical accession number.
033 – 12. The number of military and civilian personnel assigned to the laboratory by AFSC classification, as of the last day of the reporting period.

034 – 1. (1) Expenses (supplies, base operations), (2) personnel utilization (civilian and military), and (3) workload statistics (performance and assignment factors).
034 – 2. (1) Collecting data, (2) compiling and formatting data, and (3) producing management information reports.
034 – 3. (1) Pathology (the medical laboratory), (2) Radiology, and (3) Pharmacy.
034 – 4. (1) Automated Source Data Maintenance (ASDM), and (2) Expense Assignment System Preprocessor (EASPP).
034 – 5. To reduce the staff hours that medical personnel must spend in recording UCA information and preparing the required reports.
034 – 6. At work stations located in their department.
034 – 7. (1) The information will accumulate to produce daily, monthly, and quarterly reports; (2) manual tallying of statistics each quarter will be eliminated; and (3) the College of American Pathologists report data will be available on a timely basis.

035 – 1. The College of American Pathologist Inspection and Accreditation Program was developed with the primary objective of improving the quality of clinical laboratory services and ensuring the accuracy and reliability of test results.
035 – 2. It involves the performance of personnel, equipment, and materials which are observed and recorded in some systematic way.
035 – 3. It assures a thorough examination in all areas through the use of the *Inspection Checklist.*
035 – 4. The program is arranged to protect the patient whose doctor orders medical tests, and gives assurance that these findings were reliable and that tests were carried out by proper methods and qualified personnel.
035 – 5. The successful participation in an inter-laboratory comparison testing program.
035 – 6. It provides the setting for an exchange of knowledge that is beneficial for both the laboratory being reviewed and the reviewer.
035 – 7. It is advised on ways to correct deficiencies and encouraged to reapply for inspection when an acceptable standard of quality is achieved.

036 – 1. All stateside Class A, B, C, and D and overseas class A, B, and C laboratories.
036 – 2. Central procurement as authorized by the Surgeon General's office.
036 – 3. AFR 160–32, *Clinical Laboratory Classification and Capabilities.*
036 – 4. To be constructive rather than restrictive.
036 – 5. How well does this facility serve the patient under the prevailing local conditions.
036 – 6. Deficiencies that must be corrected before accreditation can be granted.
036 – 7. The deficiencies that are considered important in the management of an outstanding laboratory service and should be corrected if possible.

037 – 1. (1) b.
(2) f.
(3) d,e.
(4) c.
(5) b.
(6) b.
(7) a.
(8) a.
(9) c.
(10) i.
(11) e.
(12) d.
(13) e.
(14) j.
(15) j.

CHAPTER 4

038 – 1. Neutrons and protons.
038 – 2. The smallest particle of an element capable of taking part in a chemical change.
038 – 3. The smallest particle of an element or compound having all its chemical and physical properties.
038 – 4. It contains two atoms of the element.
038 – 5. Diatomic.
038 – 6. a. 4 of phosphorus (P) and 10 of oxygen (O) = 14 atoms.
b. 2 of hydrogen (H), 1 of sulfur (S), and 4 of oxygen (O) = 7 atoms.
c. 1 of sodium (Na) and 1 of chlorine (Cl) = 2 atoms.
038 – 7. Positive charge; 1 atomic mass unit.
038 – 8. No electrical charge; stability to the atom.
038 – 9. A negative charge.
038 – 10. Electron shells or energy levels.
038 – 11. It is responsible for determining how an atom will react during a chemical change.

039 – 1. 18.
039 – 2. Formula: Maximum number of $e-$ in the n shell $= 2n^2$.

$$e- = 2n^2$$
$$= 2 \times (4)^2$$
$$= 32$$

039 – 3. 4 (fig. 4-5).
039 – 4. The valence shell.
039 – 5. Valence electrons; they are responsible for an atom's chemical properties.
039 – 6. Ions.
039 – 7. Chemical; different.
039 – 8. The number of protons found in the nucleus.
039 – 9. They are the same or equal.
039 – 10. The atomic mass unit is merely the sum of the number of protons plus neutrons.
039 – 11. Isotopes are atoms of the same element having the same atomic number but different atomic masses.
039 – 12. The atomic weight of an element is defined as its weight relative to that of the carbon 12 isotopes.
039 – 13. amu; grams.
039 – 14. It consists of a letter, or a pair of letters, representing one atom of an element. It stands for the element itself or one atomic weight of the element.

040 – 1. Compounds represent elements that have been united chemically, whereas mixtures are united physically and can be physically separated.
040 – 2. A mixture.
040 – 3. The quantity of one element needed to combine with another element is a fixed ratio by weight for any given compound.
040 – 4. Compounds are formed from elements by bonding when an atom fills its valence shell. The valence shell is said to be "complete" or "satisfied" when it has either zero or eight electrons in it.
040 – 5. Gaining, losing, or sharing electrons.
040 – 6. Valence is the combining capacity of an element.
040 – 7. The attraction between oppositely charged ions.
040 – 8. Zero.
040 – 9. 0 (zero).
040 – 10. The K shell, which is satisfied with only two electrons since this is the maximum number it can hold.

041 – 1. (1) c, e.
(2) b.
(3) d.
(4) a.
(5) f.
(6) g.

042 – 1. $CaCl_2$.
042 – 2. CuI_2.
042 – 3. $Fe_2(SO_4)_3$.
042 – 4. $Ba(OH)_2$.
042 – 5. $Hg(NO_3)_2$.
042 – 6. $CaCO_3$, Na_2SO_4.
042 – 7. T.
042 – 8. T.
042 – 9. T.
042 – 10. F. They may form new radicals by covalently bonding oxygen to them.

043 – 1. (1) c, f.
(2) a.
(3) e.
(4) d.
(5) c.
(6) b, c.

044 – 1. Because of the fact that in a reaction matter is neither created nor destroyed.
044 – 2. (1) The total number of atoms of each element on the left must equal the total number of atoms of each element on the right; and (2) the net charge on the left must equal the net charge on the right.
044 – 3. Light, pressure, temperature, and catalysts.
044 – 4. A system in equilibrium will react to a stress by establishing a new equilibrium.
044 – 5. In the direction that absorbs heat.
044 – 6. Some reactions are accelerated by the addition of light energy.
044 – 7. Gases.
044 – 8. A substance which speeds up or slows down a chemical reaction without being changed itself.
044 – 9. Enzymes.
044 – 10. It is the formula for the equilibrium constant when two reactants, *A* and *B*, in a reversible reaction form products *C* and *D*.
044 – 11. Molar concentrations.

045 – 1. (1) a.
(2) b.
(3) h.
(4) i.
(5) c.
(6) d.
(7) e.
(8) g.
(9) f.
(10) j.
(11) k.
(12) l.

046 – 1. (1) i.
(2) g.
(3) j.
(4) c.
(5) d.
(6) a.
(7) f.
(8) e.
(9) b.
(10) f, k.

CHAPTER 5

047 - 1.
(1) e.
(2) f.
(3) m.
(4) b.
(5) i.
(6) k.
(7) c.
(8) j.
(9) l.
(10) a.
(11) h.
(12) d.
(13) g.

048 - 1. 10 ml.

048 - 2. Use formula #2 in Appendix G.

$$\frac{15 \times 500}{100} = 75 \text{ ml}$$

048 - 3. Add 50 ml of ethanol to 50 ml of a desired diluent.

048 - 4. 25.5 gm. Use formula #1, Summary of General Formulae in Appendix G.

$$\frac{0.85\% \times 3000}{100} = 25.5 \text{ gm}$$

25.5 gm q.s. to 3000 ml with water.

049 - 1. First find the gram molecular weight of NaOH by adding the atomic weights from the periodic table for each atom in the compound.

$$\begin{array}{r} 22.997 \\ +\ 16.000 \\ +\ 1.008 \\ \hline +\ 40.005 \end{array}$$

Thus, 40.005 is the gram molecular weight. To make a 2M solution you would dissolve twice this weight or 80.0 gm in 1 L of water. Since 500 ml is 1/2 L, you can divide the weight needed for a whole liter by 2, giving 40 gm to be dissolved in 500 cm^3 of water or use formula #5 in Appendix G.

$$\frac{40.0 \times 2 \times 500}{1000} = 40 \text{ gm q.s. } 500 \text{ ml}$$

049 - 2. Find the gram equivalent weight of H_2SO_4 = 49 gms. Use the formula given for percent solutions to determine the volume of a concentrated reagent which contains a desired weight of a pure compound.

$$\frac{49}{1.834} \times \frac{100}{98} \times \frac{4900}{179.7} = 27.26$$

Using formula #7 Appendix G:

$$\frac{27.26 \times 2 \times 1000}{1000} = 54.5 \text{ ml of concentrated reagent}$$

049 - 3. +3, because total positive valence = 3.

049 - 4. $\frac{98 \times 1 \times 200}{2 \times 1000} = 9.8$ gm

049 - 5. The molecular weight of HCl is 36.47

$$\frac{36.47 \times 0.001 \times 200}{1000} = 0.007 \text{ gm}$$

0.007 × 2.3 = .016 ml

049 - 6. The molecular weight of HNO_3 = 63.02 Determine the volume of a concentrated reagent which contains a desired weight of a pure compound. Then use formula #6 Appendix G.

$$\frac{63.02}{1.4} \times \frac{100}{70} = \frac{6302}{98} = 64.3 \text{ gm}$$

$$\frac{64 \times 0.1 \times 500}{1000} = \frac{3200}{1000} = 3.2 \text{ ml}$$

049 - 7. The molecular weight of H_2SO_4 is 98

$$\frac{98}{1.84} \times \frac{100}{95} = \frac{9800}{174.8} = 56.06 \text{ gm}$$

$$\frac{56.06 \times 2 \times 100}{1000} = 11.2 \text{ ml}$$

049 - 8. The equivalent weight of HCl is 36.47

$$\frac{36.47}{1.19} \times \frac{100}{38} = \frac{3647}{45.22} = 80.65$$

$$\frac{80.65 \times 1.5 \times 500}{1 \times 1000} = 60.48 \text{ ml or } 60.5 \text{ ml}$$

050 - 1. $85x = 100 \text{ X } 65$
$x = 76.5$ ml

050 - 2. $(600)(15) = 3x$
$x = 3000$ ml

051 - 1. $\text{mEQ/L} = \frac{\text{mg/dl} \times 10}{\text{Atomic wt } \surd}$ Atomic wt: 40 Valence: 2

$$= \frac{8 \times 10}{20} = \frac{80}{20} = 4.0 \text{ mEq/L}$$

051 - 2. $\text{M mol/L} = \frac{\text{mg/dl} \times 10}{\text{Atomic wt.}}$

$$\frac{8 \times 10}{40} = \frac{80}{40} = 2.0 \text{ M mol/L}$$

051 - 3. Atomic wt 30.9.

$$\frac{4.0 \times 10}{30.9} = \frac{40}{30.9} = 1.3 \text{ M mol/L}$$

051 - 4. $\frac{2.5 \times 10}{30.9} = 0.8$

$$\frac{4.8 \times 10}{30.9} = 1.55$$

Adult normal values: 0.8 to 1.55 M mol/L

052 - 1.
(1) h.
(2) e.

(3) f.
(4) b.
(5) d.
(6) g.
(7) a.
(8) a.
(9) f.
(10) j.
(11) i.

053 – 1. $\frac{\text{acid}}{40x} = \frac{\text{base}}{80 \times 0.5}$

$x = 1n$

053 – 2. $\frac{\text{NaOH}}{10x} = \frac{H_2SO_4}{5 \times 1N}$

$x = 0.5N$

053 – 3. $\frac{\text{base}}{25x} = \frac{HNO_3}{5 \times 10}$

$x = 2N$
(The water is of no significance.)

053 – 4.

base	*acid*	
6.30	$6.3x = 5 \times 0.1$	$x = .079N$
6.35		$x = .079N$
6.25		$x = .080N$
	Average: .079N or .08N	

053 – 5.

acid *base*
$20x = 30 \times 0.5$
$x = 0.75N$

053 – 6. Remember that a 1 molar solution of sulfuric acid H_2SO_4 represents a 2– normal solution. The quantity of base used is not pertinent to the problem. Therefore:

$+ 0.1M\ H_2SO_4 = 0.2N\ H_2SO_4$

053 – 7. You must recall that 1 molar solution of sulfuric acid H_2SO_4 represents a 2 normal solution. Therefore:

$1M\ H_2SO_4 = 2N\ H_2SO_4$

thus: $10x = 5 \times 2$
$x = 1N$

CHAPTER 6

054 – 1. Wavelength, frequency, and energy.
054 – 2. Frequency and energy.
054 – 3. Wavelength.
054 – 4. The actual wavelength.
054 – 5. A spectrum or rainbow.
054 – 6. In nanometers (1 nm equals 1×10^{-6}m).
054 – 7. 723 nm to 397 nm.
054 – 8. 397 nm.

055 – 1. Colorimeters use filters for isolation of part of the spectrum, whereas spectrophotometers use gratings and prisms.
055 – 2. It is the measurement of the light-transmitting power of a solution in order to determine the concentration of light-absorbing substances present within.
055 – 3. They will absorb light energy.
055 – 4. Light.
055 – 5. Number of colored molecules or ions in the path of light.

056 – 1. Light absorption.
056 – 2. Power supply, exciter lamp, monochromator, sample holder, photodectector, and readout.
056 – 3. Relatively high sensitivity, ease of rapid measurement, and relatively high degree of specificity.
056 – 4. Absorb energy of a specific wavelength.

057 – (1) i.
(2) c.
(3) d.
(4) d.
(5) e.
(6) f.
(7) m.
(8) n.
(9) g, h.
(10) g, h.
(11) g, h.
(12) h.
(13) h.
(14) a.
(15) a.
(16) b.
(17) b.
(18) i.

058 – 1. The concentration.
058 – 2. It becomes invalid or does not apply.
058 – 3. Repeat the entire procedure using a dilution of the original specimen.
058 – 4. Light rays are scattered by suspended particles.
058 – 5. By filtration or centrifugation.
058 – 6. The amount of standard in 1 ml times the number of milliliters used in the procedure.
058 – 7. $V = \frac{\text{ml serum or actual specimen} \times \text{ml diluted specimen used}}{\text{total volume of diluted specimen}}$
058 – 8. $C_s = 0.05\ mg/ml \times 3\ ml$
$C_s = 0.15\ mg$
058 – 9. $V = \frac{1 \times 2}{10}$

$V = 0.2$

059 – 1. To A (absorbance) or %T (percent transmittance) and is necessary in quantitative work where the amount of absorption has to be calculated.
059 – 2. Semilogarithmic graph paper.
059 – 3. It is necessary to reconstruct the entire curve in order to determine its proper shape.
059 – 4. Save time and simplify calculations.
059 – 5. They are synonymous.
059 – 6. Optical density is logarithmically proportional to transmittance.
059 – 7. Optical density.
059 – 8. A spectral-absorbance curve enables you to obtain the wavelength at which maximum absorption takes place with a given solution.
059 – 9. An inverted curve results, but all of the principles are the same.

060 – 1. Each day.
060 – 2. Whenever lamps are changed or instruments are repaired or moved about.
060 – 3. Linearity.
060 – 4. Purchased, accurately prepared standards.
060 – 5. They are recorded and kept permanently.
060 – 6. The peak width at 50 %T.
060 – 7. Below 20 %T and over 60 %T.
060 – 8. It should correspond fairly closely to the band width claimed by the manufacturer.
060 – 9. Reflection within the instrument, light from the next higher order spectrum, or room light reaching the detector.
060 – 10. Blocking filters.

061 – 1. (1) a.
(2) c.
(3) e.
(4) g.
(5) g.
(6) h.
(7) f.
(8) d.
(9) b.
(10) d.

062 − 1. Poor maintenance and operator error.
062 − 2. From 335 to 700 nm.
062 − 3. 8 nm.
062 − 4. Review operator's manual to become familiar with basic operation principles.
062 − 5. 15 minutes.
062 − 6. Use a vacuum regulator.
062 − 7. Set the CON−ABS−ACC control to ABS.
062 − 8. Make sure that the sample inlet tubing is well below the surface of the sample material without touching the bottom of the sample container.
062 − 9. Insufficient sample intake.
062 − 10. Introduce the reference blank and zero the instrument.

063 − 1. Serum, urine, or other specimen is vaporized by aspiration into a flame to produce certain colors of light. Color that is characteristic of the light emitted by the constituent being measured is passed through a filter to a photo-electric cell, where its intensity is measured.
063 − 2. The selection of a spectral line of strong enough intensity to provide adequate sensitivity and freedom from other interfering lines at or near the selected wavelength.
063 − 3. The light given off may not always provide adequate sensitivity for analysis.
063 − 4. Sodium 140 mEq/L, potassium 5.0 mEq/L.
063 − 5. Cesium and lithium.
063 − 6. Internal standards such as lithium or cesium act to absorb the radiation and buffer the potassium atoms from the effects of mutual excitation.
063 − 7. The regulator.
063 − 8. The atomizer and the flame.
063 − 9. To break up the solution into fine droplets so that the atoms will absorb heat energy from the flame and become excited.
063 − 10. Sodium, potassium, and lithium.
063 − 11. An unsteady flame, dirty glassware, clogged aspirator, and hemolysis.

064 − 1. Atomic absorption measures the absorption of light by unexcited atoms. This method is used to determine a number of metallic elements in low concentrations in biologic material.
064 − 2. In flame photometry, one measures the light emitted by the small fraction of the sample atoms in the flame that are excited to emit their characteristic radiation, whereas in atomic absorption, one measures the absorption of light by the unexcited atoms.
064 − 3. Calcium, manganese, sodium, potassium, chromium, mercury, lithium, nickel, bismuth, cobalt, and manganese.
064 − 4. In flame photometry, the presence of other elements may cause some interference with elements being determined. For example, phosphate causes some interference with calcium determinations.

065 − 1. Some chemical compounds have the property of absorbing light energy and then remitting some of this energy in a longer wavelength than when absorbed. The longer wavelength results in less energy remitted.
065 − 2. The ultraviolet or near ultraviolet wavelength and a range of 250–400 nm.
065 − 3. Longer; visible.
065 − 4. The introduction of a set of filters or a monochromator before and after the cell to isolate the emitted light.
065 − 5. A mercury lamp or xenon lamp.
065 − 6. Increased sensitivity.
065 − 7. They cause false scattering of light.
065 − 8. It causes a decreasing fluorescence.

066 − 1. Hydrogen ion (H^+) pH, sodium (Na^+), potassium (K^+), calcium (Ca^{++}), fluoride (F^-), and chloride ($C1^-$).
066 − 2. They develop an electrical potential that is a function of the amount of that ion present.
066 − 3. Two.
066 − 4. Chloride and carbon dioxide.
066 − 5. The contents of the glass membrane itself.
066 − 6. Highly selective solid state electrodes composed of AgC1 (silver chloride).

CHAPTER 7

067 − 1. Instruments that continuously pump reagent through tubing and coils to form a flowing stream and continuously pump samples into that stream.
067 − 2. A peristaltic pump.
067 − 3. It is composed of steel roller rods that compress several pieces of plastic tubing by rolling on them.
067 − 4. The inner diameter of the tubing employed.
067 − 5. Small air bubbles injected into the sample and reagent streams at strategic points.
067 − 6. This creates a tumbling motion in each segment.
067 − 7. A dialysis membrane.
067 − 8. The length and inner diameter.

068 − 1. The discrete analysis involves the treatment and measurement of samples in individual containers, whereas the continuous flow system employs a proportioning system for measuring samples and reagents.
068 − 2. Samples are pipetted by the instrument into reaction tubes.
068 − 3. Reaction tubes then move through various stations where more reagents are added in addition to the sequential process of mixing and heating.
068 − 4. Cuvette.
068 − 5. Into separate compartments of a transfer disk.
068 − 6. By centrifugal force.
068 − 7. As the cuvettes pass through the vertical light beam of a spectrophotometer.

069 − 1. A dialyzer or resin column.
069 − 2. Recorder, printer, or computer.
069 − 3. Conveyer travel.
069 − 4. To insure continuing accuracy of results.
069 − 5. Reproducibility.
069 − 6. Only if the instrument or system is operating in a sound manner.
069 − 7. Because of the automated characteristics of the machine.
069 − 8. Due to their uniform objectivity, instruments cannot exercise the judgment expected from an experienced technician.
069 − 9. Backup procedures.

070 − 1. The minimum time required to obtain a result after the initial sampling of specimen.
070 − 2. The maximum number of tests that can be processed in an hour.
070 − 3. Instruments that perform the same test simultaneously on all samples.
070 − 4. (1) a.
(2) c.
(3) c.
(4) a, c.
(5) d.
(6) d.
(7) b.
(8) b.
(9) e.
(10) e.
(11) e.
(12) d, e, f, h.
(13) h.
(14) h.
(15) g.
(16) f.
(17) f.
(18) a, c.

071 − 1. Instrument control.
071 − 2. They manage many functions such as instrument operations, data collection, and processing, all at the same time.
071 − 3. Laboratory information systems (LIS).
071 − 4. (1) Perform repetitive tasks.
(2) Perform complex calculations.
(3) Collect, organize, store, and distribute large amounts of data.
(4) Operate machines in a highly reproducible manner.

071 − 5. Aspiration of sample and reagents, collection of data, calculation, and printout results.

071 – 6. The Du Pont ACA–III.
071 – 7. The computer simply compares the current value with the acceptable range supplied by the user. If the result is outside this range, it is flagged so that the user may choose to reject the run.
071 – 8. They are sophisticated computer systems designed to handle the problems of collecting, organizing, and reporting data from the clinical laboratory.

CHAPTER 8

072 – 1. The median cubital vein. It is large, close to the skin, and least painful to the patient.
072 – 2. These sites are not recommended for use in patients with diabetes or a cardiovascular or circulatory problem.
072 – 3. The count might be affected by lymphostasis (stoppage of lymph flow).
072 – 4. The specimen will be diluted. Test results will be erroneous and misleading to the physician.
072 – 5. Below the i.v. site when a specified procedure is followed.
072 – 6. Oncology patients, leukemia patients, patients with constant i,v, therapy, extremely obese patients, babies and children, and cardiac patients.
072 – 7. Bounce, direction of vein, size of needle, and depth.
072 – 8. The median cubital vein; it is usually bigger, anchored better, and bruises less.

073 – 1. Concentrations in whole blood exhibit a tendency to vary, depending on the proportion of erythrocytes. Concentrations in serum or plasma remain constant.
073 – 2. Heparinized whole blood. The main substance of interest is found chiefly in red cells.
073 – 3. Whole blood.
073 – 4. Flouride.

074 – 1. They chelate calcium.
074 – 2. The molarity necessary for effective coagulation and the ability to cause water loss or gain from the cells with its associated change in plasma components.
074 – 3. Sodium or potassium salts of citrate or oxalate causes a loss of cell water and dilution of plasma, while the use of the ammonium salt causes gain in the cell water.
074 – 4. Potassium and ammonium salts.
074 – 5. EDTA.
074 – 6. It inhibits thrombin and other stages of clothing activation.
074 – 7. Heparin.
074 – 8. They produce inhibitory actions on certain enzymes such as involved in glycolysis.
074 – 9. Low or reduced results. Flouride will inhibit the action of glucose oxidase.

075 – 1. Cortisol, iron, estriol, catecholamines, corticosteroids, glucose, and triglycerides.
075 – 2. Total protein, albumin, lipids, iron, calcium, and enzymes.
075 – 3. The movement of water out of the intravascular compartment upon standing probably causes this effect.
075 – 4. Pooling of blood above the constriction of the tourniquet; cause by the prolonged use of the tourniquet.
075 – 5. Use of too large or too small a needle, moisture in a syringe, vigorous mixing of blood, rapid expansion of the blood in the tube, or the separation process.
075 – 6. A false increase in serum concentration of constituents that are present in high concentration within the red cells, and a dilution effect on the serum constituents that exist at lower concentrations in serum.
075 – 7. Potassium and enzymes.
075 – 8. Catecholamine level and blood-gas results.

076 – 1. Phosphorus.
076 – 2. Increased; decreased in both glucose and proteins.
076 – 3. Accurate test results.
076 – 4. Label and store in refrigerator at 4°C to 6°C until analyzed or freeze at −20°C if analysis will be delayed more than 4 hours.
076 – 5. Potassium, calcium, and glucose.
076 – 6. To reduce the potential for leakage of constituents from the cells through the filter into the serum.
076 – 7. It should be labeled with all of the data necessary to identify it, the date drawn, and preparation details.

077 – 1. Be certain that the tubes are labeled.
077 – 2. Tube number 2.
077 – 3. Serum will remain in the supernatant after centrifugation. This fluid could not be used for protein determination.
077 – 4. Chloride.
077 – 5. Bacteria utilize the glucose in their metabolism.

078 – 1. (1) f.
(2) a.
(3) d.
(4) c, d.
(5) e.
(6) a.
(7) b.
(8) f.

079 – 1. T.
079 – 2. F. The lack of contaminants in a primary standard is not completely desirable because a pure standard does not react entirely as a test specimen which contains inhibitors or other contaminants.
079 – 3. T.
079 – 4. F. They are not chemically identical to the substance assayed, but they are related through some physical or chemical property, which makes them useful as standards.
079 – 5. T.
079 – 6. F. An accurate standard may be used in a manner which results in completely unreliable results.
079 – 7. F. The quality of the standard will be invalidated.
079 – 8. F. A curve should be checked each time the procedure is performed because conditions may have changed since the curve was established.
079 – 9. T.
079 – 10. F. The range of value for a control ideally should be close to that of the specimen being assayed.
079 – 11. F. A Control is not necessarily assigned a definite value.
079 – 12. T.
079 – 13. T.
079 – 14. F. The control does not automatically become a suitable standard. A solution that does not contain an exact known amount of the constituent in a suitable solvent falls short of the definition reserved for a standard.

080 – 1. (1) a.
(2) g.
(3) b.
(4) b.
(5) c.
(6) e.
(7) e.
(8) d.
(9) d.
(10) e.

081 – 1. A digit that denotes the amount of the quantity in the spot in which it stands.
081 – 2. a. 675—six hundreds, seven tens, and five units.
b. 067—only digits in tens and units are significant figures.
c. 6750—all figures are significant.
081 – 3. No.
081 – 4. No, they merely place the decimal point in its correct position.
081 – 5. Yes, they both are. The number is greater than 20 and not quite 21. It is closer to 21.510 than 20.509 or 20.511. The position of the zeros verifies this fact.
081 – 6. As one significant figure or where it represents a measured value. For example, blood glucose results are reported as 100 mg/dl and not 100.55 mg/dl. .55 does not represent a truly measured value. A chloride value is reported as 105 mEq/L instead of 105.55 mEq/L. .55 does not represent a measured value. One significant figure is sufficient, 105 mEq/L.

082 – 1. The technician.
082 – 2. By monitoring all factors associated with standardization and instrument performance.

082 – 3. Must be aware of problems in areas for which he or she is assigned and correct them.

082 – 4.
- a. Provide clearly written procedures for all analyses in order to reduce variation in technics.
- b. Provide written instructions concerning steps to take when the QC system indicates a problem.
- c. Help analyze trends.
- d. Choose samples for repeat analysis.

082 – 5. To insure that problems are identified and that corrective action is taken.

083 – 1. Measurement of conductivity.

083 – 2. "Date received" and, when first opened, "Date opened."

083 – 3.
- a. Name of the test for which the reagent was prepared.
- b. Reagent name.
- c. Date of preparation with initials of the person who prepared it.
- d. Date of reagent check with initials.
- e. Date of expiration.
- f. Special instructions or warnings.

083 – 4. Each has a separate and important function.

083 – 5. It is used to standardize the method or instrument.

083 – 6. To maintain a year-in, year-out consistent level of analytical accuracy.

083 – 7. As soon as they are received.

083 – 8. Record all solution changes, all instrument repairs, and all maintenance procedures.

083 – 9. Joint Committee on Accreditation of Hospitals (JCAH) and the College of American Pathologists (CAP).

083 – 10. Joining appropriate professional societies, reading technical journals and books, attending scientific meetings, and taking continuing education courses.

084 – 1. It measures variables, including reagent and method reliability, technician skill, and instrumentation.

084 – 2. Total allowance variation.

084 – 3. By calculating the dispersion of values for a test on either side of the average value.

084 – 4. Usually two.

084 – 5. The sum of the squared difference from the average.

084 – 6. 34 percent. Assuming random distribution and a statistically adequate number of tests, one-half of ± 1σ units (68 percent of the values) is −1σ, or 34 percent of the values.

085 – 1. There were 21 replicate analysis for glucose. The sum of all values for glucose was 2,457. The average, or mean, value is obtained by dividing the sum of all values by the number (n) of determinations.

$$x = \frac{\text{sum}}{n} = \frac{2457}{21} = 117$$

085 – 2. You must calculate the differences from the average value (d) and the square of this difference (d^2) for each test value. For instance, the first value was 118 mg/dl. The difference between 118 and 117 is 1, so this is the first number in the (d) column. The difference squared (d^2) column or (1^2) is also 1. The second specimen (115) differs from the average (117) be 2, so the second value in column (d) is 2 and in the (d^2) column the second value is (2^2), which is 4. Check your calculations with the answers below and correct any mistakes.

Specimen	Test Results	(d)	(d^2)
1	118	1	1
2	115	2	4
3	111	6	36
4	114	3	9
5	117	0	
6	117	0	
7	120	3	9
8	116	1	1
9	112	5	25
10	117	0	
11	122	5	25
12	119	2	4
13	117	0	
14	117	0	
15	121	4	16
16	121	4	16
17	121	4	16
18	117	0	
19	111	6	36
20	115	2	4
21	119	2	4
sum =	2457		d^2 = 206

$$\frac{\text{sum}}{n} = 117 = x$$

The formula for calculating standard deviation is:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n - 1}}$$

The number of tests (n) is known. You have just calculated the d^2 values. Add the d^2 column of figures to obtain the sum of d^2 or Σd^2. Substitute n and Σd^2 values in the standard deviation formula and solve for σ. From your calculations you should have obtained the values stated below:

$$\sigma = \sqrt{\frac{\Sigma d^2}{n \cdot - 1}} = \sqrt{\frac{206}{21 - 1}}$$

$$\sqrt{\frac{206}{20}} = \sqrt{10.3}$$

$$\sigma = 3.2 \text{ mg/dl}$$

Figure A–1. Objective 085, exercise 2.

085 – 3. Confidence limits establish the total allowable variation of a method. Since two standard deviations are given as allowable variation, 2 × the standard deviation value (+ 3.2) = total allowable variations of 6.4 mg/dl. This allowable variation is valid only at the mean level of concentration (117 mg/dl + 6.2 mg/dl or from 111 mg/dl to 123 mg/dl).

085 – 4.

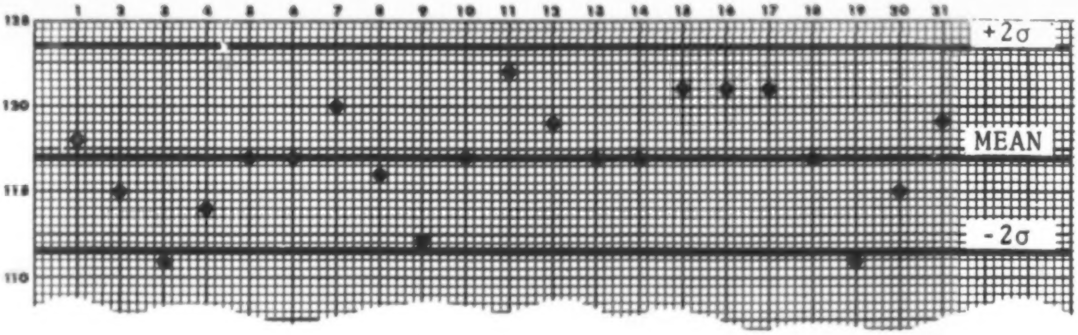


Figure A–2. Objective 085, exercise 4.

085 – 5. Recorded.

085 – 6. $\frac{159}{19} = \sqrt{8.37} = 2.89$

086 – 1. The USAF Graduate Evaluation Program serves to provide feedback data to validate the effectiveness of training in your specialty.

086 – 2. The used of AF Form 1284, Training Quality Report, and ATC Education and Training Programs.

086 – 3. The graduate, the immediate supervisor, and others having knowledge of the graduate's performance.

086 – 4. The graduates and their supervisors.

086 – 5. Specific questions in the last volume review exercise booklet and statistics on test questions from the volume review exercises and course exam.

085 – 6. Write or call the author using the AUTOVON 736–2809, between 0700 and 1600 (CT).

1985-631-028/20481 AUGAFS,AL(854627)1300

STOP- 1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.
2. USE NUMBER 2 PENCIL ONLY.

EXTENSION COURSE INSTITUTE
VOLUME REVIEW EXERCISE

90411 01 24

INTRODUCTION TO MEDICAL LABORATORY ADMINISTRATION AND TO CLINICAL CHEMISTRY

Carefully read the following:

DO's:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that item numbers on answer sheet are sequential in each column.
3. Use a medium sharp #2 black lead pencil for marking answer sheet.
4. Write the correct answer in the margin at the left of the item. (When you review for the course examination, you can cover *your* answers with a strip of paper and then check your review answers against your original choices.) After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if at all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor. If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'Ts:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than a #2 black lead pencil.

NOTE: **NUMBERED LEARNING OBJECTIVE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE.** In parenthesis after each item number on the VRE is the *Learning Objective Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Learning Objectives* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual* VRE *items you missed*. Go to the VRE booklet and locate the *Learning Objective Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

MULTIPLE CHOICE

Note to Student: Consider all choices carefully and select the *best* answer to each question.

1. (001) Which listed factor is most important in determining the amount and variety of service a medical laboratory provides?

   a. The size of the medical facility which it supports.
   b. The interest that the hospital commander takes in the laboratory.
   c. The ingenuity of the laboratory superintendent and section supervisors.
   d. The interest that the pathologist and laboratory officer take in their department.

2. (002) The skilled levels may be designated by the numbers 1, 3, 5, 7, or 9. What specific skilled level does the 7 represent?

   a. Semiskilled.
   b. Skilled.
   c. Advanced.
   d. Seven.

3. (003) Which listed high school courses are mandatory for an airman to enter the medical laboratory career field?

   a. Algebra and biology.
   b. Chemistry and algebra.
   c. Chemistry and zoology.
   d. Biology and zoology.

4. (004) In order to advance to the 7-level of AFSC 92470, all of the following criteria must be fulfilled *except*

   a. completion of CDC Course 90411, 90412, and 90413.
   b. 12 months' OJT.
   c. completion of PME-Phase II.
   d. completion of ECI Course 9, Command NCO Academy Course.

5. (005) In order to enter course ABR92430 by proficiency advancement, what basic experience must qualify an airman?

   a. Prior civilian experience or military occupational experience.
   b. Advanced college courses in algebra and biology.
   c. Advanced college courses in physics and biology.
   d. Prior civilian experience in histopathology or cytology.

6. (006) If a technician has failed to qualify for the 7-level within the specified time period allowed, a classification board may recommend all of the following actions *except*

   a. withdrawal of AFSC 92450 and return to duty in an awarded AFSC.
   b. withdrawal of AFSC 92450 and retrain into another AFSC more compatible with capabilities.
   c. removal from training.
   d. continued training until the technician qualifies for the 7-level.

7. (007) The AFSC 92499 may be awarded to a master sergeant after completion of all the following criteria *except*

   a. minimum of 12 months in grade.
   b. possess a seven level.
   c. has completed the Senior NCO Academy Associate Program.
   d. passed the USAF Supervisory Examination.

8. (008) Which of the following duties and responsibilities are *least* considered to be those for a Medical Laboratory Manager, CEM 92400?

   a. Interprets and enforces applicable directives.
   b. Recommends actions to improve efficiency of laboratory operations.
   c. Recommends actions to improve the efficiency of the chemistry section quality control program.
   d. Develops plans regarding procurement and maintenance of laboratory facilities and equipment.

9. (009) Under WAPS, an individual scored the following APR points for the last 5 years: 7, 9, 8, 9, 8, 8, 9, 9, and 9. Compute his or her APR points.

   a. 8.4.
   b. 76.0.
   c. 114.0.
   d. 127.0.

10. (010) Which of the following duties would most likely be accomplished by a medical laboratory specialist and/or a medical laboratory technician?

    a. Plans and conducts on-the-job training.
    b. Accomplishes standardized qualitative and quantitative evaluation of erythrocytes.
    c. Accomplishes general laboratory duties.
    d. Assists in blood bank duties.

11. (011) Investment medical equipment includes items that are expected to last 5 years or longer and which cost at least

    a. $500.
    b. $1,000.
    c. $2,000.
    d. $3,000.

12. (012) Which of the following Federal supply catalogs identifies instruments and laboratory equipment?

    a. FSC 6505.
    b. FSC 6500.
    c. FSC 6515.
    d. FSC 6600.

13. (013) What form should you use for ordering nonstandard items which cost less than $300?

    a. DD Form 1348-6.
    b. AF Form 115a.
    c. AF Form 601.
    d. AF Form 1517.

14. (013) When submitting AF Form 601 with equipment that is deemed unserviceable what two conditions should be mentioned?

    a. The condition code as determined by the MER technician and the life expectancy.
    b. The replacement item and the maintenance cost.
    c. The condition code as determined by the MER technician and the maintenance cost.
    d. The replacement item and the life expectancy.

15. (014) You are authorized a maximum supply level of

    a. 1 week.
    b. 2 weeks.
    c. 3 weeks.
    d. 4 weeks.

16. (014) If you stockpile an item and do not order regularly, you might influence the medical supply level

    a. to be reduced.
    b. to be increased.
    c. to decrease to zero.
    d. in any of the ways above.

17. (015) Authorization for medical equipment items may be found in the

a. table of allowances.
b. latest supply catalog.
c. computerized shopping guide.
d. index of indexes for AF standard publications.

18. (015) When budgeting for supplies and equipment, how much should you add to estimates to compensate for inflation and price increases?

a. 5 percent.
b. 10 percent.
c. 15 percent.
d. 20 percent.

19. (016) You admit pecuniary liability for an Air Force medical equipment item which cost $1,000. What action must be taken?

a. Pay $1,000 in cash.
b. AF Form 198, Report of Survey for Air Force Property, must be prepared.
c. DD Form 362, Statement of Charges, must be prepared.
d. At your discretion, you may either pay in cash or you may initiate DD Form 362, Statement of Charges.

20. (017) Reporting and inspections of unsafe or unhealthful working conditions may be directly made to all of the following *except*

a. base inspector general.
b. laboratory supervisor.
c. hospital safety officer.
d. base bioenvironmental engineer.

21. (018) When safety is practiced in the laboratory, the *prime* concern is for the

a. technician and his equipment.
b. technician and his coworkers.
c. patients and the laboratory equipment.
d. patients and laboratory coworkers.

22. (019) When used in the laboratory, which of the following items is *most likely* to constitute a serious hazard and will absorb certain solvents?

a. Cosmetics.
b. Contact lenses.
c. Wrist jewelry (rings).
d. Broken glassware.

23. (020) When preparing reagents using an acid and water, in what order should they added and how?

a. Add acid to water; allow acid to run down the side of container and mix slowly.
b. Add water to acid; allow water to run down the side of container and mix rapidly.
c. Add water to acid; allow water to run down the side of container and mix slowly.
d. Add acid water; allow acid to run down the side of container and mix rapidly.

24. (021) What is the medically recommended method of emergency treatment of chemical eye injuries?

a. Wash thoroughly with plain water for 15 minutes without delay.
b. Wash thoroughly with boric acid then wash with plain water for 15 minutes.
c. If acid, first neutralize, then wash for 20 minutes with plain water.
d. If alkali, first neutralize, then wash for 20 minutes with plain water.

25. (022) What form would you prepare for a report of accident in the laboratory?

a. AF Form 457, USAF Hazard Report.
b. AF Form 765, Hospital Incident Statement.
c. AF Form 711, USAF Mishap Report.
d. AF Form 711a, Ground Mishap Report.

26. (023) Standard laboratory forms appear in triplicate and are completed and routed in what manner?

a. First copy for the patient's records, second copy to the doctor, and the third copy filed in the lab.
b. First copy filed in the lab, second copy for the patient's records and third copy to the doctor.
c. First copy to the doctor, second copy put in the patient's records, and third copy in the lab files.
d. First copy to the patient's record, second copy in the lab file, and third copy to the doctor.

27. (024) If you were interested in information on Blood Bank standards, in what numerical series and general area of AFR 0-2 would you look?

a. 160 - Medical Service.
b. 161 - Aerospace Medicine.
c. 168 - Medical Administration.
d. 169 - Medical Education and Research.

28. (025) Air Force publications may be requested from what office?

a. Personnel and Administrative Services.
b. Medical Reference Library.
c. Resource Management.
d. Medical Material.

29. (026) If your laboratory maintains official files what form must be completed to show all official documentation held in your laboratory?

a. AF Form 82, Files Disposition Control Label.
b. AF FOrm 80, Files Maintenance and Disposition Plan.
c. AF Form 44, Certificate of Records.
d. AF Form 166, Annual Report of Documentation Holdings and Disposition.

30. (027) Which of the following statements *incorrectly* depicts orientation of a new lab technician?

a. Describe the function of the department and where he fits in.
b. Take plenty of time to assign the new tech to his position.
c. Provide job instruction, assistance, and give him encouragement.
d. Discuss his personal interests and make him feel he will be a useful part of the group.

31. (028) When is there a justification for reporting patient information outside the "need-to-know" group?

a. When the patient is unconscious.
b. When the patient is under age.
c. There is never a justification.
d. When the patient is your lab coworker.

32. (029) Which of the following actions will *best* contribute to providing quality laboratory service?

a. Tell your supervisors of your best work areas in the laboratory.
b. Tell all other techs of your best work area in the laboratory.
c. Take a few seconds to tell the physicians of your best work ares.
d. Take a few seconds to double check your results and repeat work when necessary.

180

33. (030) The reputation of your facility depends upon

a. the NCOIC and section supervisors.
b. the reputation of the hospital commander.
c. the reputation of the pathologist and laboratory officers.
d. your reputation as well as the reputation of every member of the staff.

34. (031) In an AUTOVON conversation, the NCOIC of the USAF Clinic at Hickam Air Force Base informed the NCOIC of Patterson Medical Center laboratory that they were increasing their manning strength and preparing for extended laboratory service. Which of the following information about the USAF Clinic facility could *most likely* have been obtained through communications monitoring?

a. Deployment, plans and programs.
b. Capabilities, equipment number and weaknesses.
c. Capabilities, equipmeat number and intentions.
d. Deployment, plans and weaknesses.

35. (032) The Clinical Laboratory Report of Patients has all of the following given primary uses *except*

a. budgeting and financial planning.
b. manpower and staffing.
c. facilities and equipment procurement.
d. equipment standardization and renovation of facilities.

36. (033) New editions of the manual containing the "unit of count" values are provided by the

a. office of the Air Force Surgeon General.
b. office of the Command Surgeon.
c. Medical Resource Management Office.
d. Air Force Consultant in Pathology.

37. (034) Which of the following is *not* not considered to be part of the three main components in the UCA operations?

a. Collecting data.
b. Compiling and formatting data.
c. Producing management information reports.
d. Producing inventory information on laboratory equipment.

38. (035) A significant feature of the College of American Pathology Inspection and Accreditation Program is that it assures a thorough examination in *all areas* through the use of the

a. Proficiency Testing.
b. Inspection Checklist.
c. Educational benefits.
d. Proficiency monitoring.

39. (036) Deficiencies that must be corrected before accreditation can be granted are represented as what Phase?

a. Phase 0.
b. Phase I.
c. Phase II.
d. Phase III.

40. (037) Which of the following units serves as the national focus for developing and applying disease prevention and control, environmental health, and health promotion and health education activities?

a. Armed Forces Institute of Pathology.
b. USAF Histopathology Centers.
c. Centers for Disease Control (CDC).
d. USAF Epidemiological Services.

41. (038) That which appears as the smallest particle of an element or compound having all its chemical and physical properties best describes

a. an atom.
b. a molecule.
c. subatomic particles.
d. an atomic mass.

42. (039) Using the formula: maximum number of e^- in the n shell = $2n^2$, what is the maximum number of electrons that will hold in the M shell if the n number is 3?

a. 6.
b. 9.
c. 18.
d. 32.

43. (040) The number of electrons an element is able to gain, lose, or share is the element's

a. oxidation.
b. valence.
c. ionization.
d. atomic number.

44. (041) Chemical bonding in which each atom donates one or more valence electrons to be shared equally by two is called

a. covalent bonding.
b. coordinate bonding.
c. coordinate covalent bonding.
d. electrovalent coordinate bonding.

45. (042) All of the following are rules for writing chemical formulas *except*

a. cation is written first.
b. drop all positive or negative signs.
c. retain the subscripts if they are numerically equal.
d. if a radical is taken more than once, enclose it in parentheses and put the subscript outside the parentheses.

46. (043) Which of the following equations represents a *single displacement reaction*?

a. $2Mg + O_2 \longrightarrow 2Mg0$.
b. $CaCO_3 \longrightarrow CaO + CO_2$.
c. $Zn + CuSO_4 \longrightarrow ZnSO_4 + Cu$.
d. $BaCl_2 + CuSO_4 \longrightarrow BaSO_4 + CuCl_2$.

47. (044) What type of catalyst is of most interest to the clinical chemist?

a. Equilibrium.
b. Concentration.
c. Enzymes.
d. Light energy.

48. (045) When acids and bases react and the proton (H^+ ion) reacts with the proton acceptor (OH^- ion) to form water the result is *best* described as

a. hydrolysis.
b. neutralization.
c. ionization.
d. oxidation.

49. (046) Select an example of a disaccharide.

a. Dextrose.
b. Fructose.
c. Sucrose.
d. Starch.

50. (047) "The amount of solute present in a given volume of solution" *best* describes

a. a solute.
b. a solvent.
c. concentration.
d. saturation.

51. (048) How many milliliters of a base are used to make 1500 ml of 25 percent V/V solution?

a. 225 milliliters.
b. 375 milliliters.
c. 175 milliliters.
d. 125 milliliters.

52. (049) How many milliliters of sulfuric acid (molecular weight 98, specific gravity 1.84 and 98.0 percent purity) are needed to prepare 500 milliliters of a 2 N solution?

a. 26.2 milliliters.
b. 27.2 milliliters.
c. 28.2 milliliters.
d. 29.2 milliliters.

53. (050) How many milliliters of a 10 percent solution of glucose are required to prepare 112 milliliters of a 5 percent solution?

a. 2.04 milliliters.
b. 5.6 milliliters.
c. 22.4 milliliters.
d. 56.0 milliliters.

54. (051) How many milliequivalents per liter are equal to 10 mg/dl of calcium (atomic wt: 40, valence: 2)?

a. 5 mEq/L.
b. 10 mEq/L.
c. 15 mEq/L.
d. 20 mEq/L.

55. (051) If the normal values for inorganic phosphorus in children is 3.5 to 6.0 mg/dl, what are the normal values in mmol/L (atomic wt: 30.9)?

a. 1.1 to 1.9 mmol/L.
b. 2.1 to 2.9 mmol/L.
c. 3.1 to 3.9 mmol/L.
d. 3.5 to 6.0 mmol.L.

56. (052) The term "pH" is taken from an expression which literally means presence of

a. hydroxyl ions.
b. hydrogen ions.
c. buffer systems.
d. indicator systems.

57. (052) A solution which resists a change in pH upon the addition of significant quantities of hydrogen or hydroxyl ions can *best* be described as

a. an acid.
b. a base.
c. a buffer.
d. an indicator.

58. (053) 20 ml of an acid will neutralize 40 ml of an 0.6 N base. What is the normality of the acid?

a. .012 N.
b. 1.2 N.
c. 112 N.
d. 12 N.

59. (054) What property determines the nature of electromagnetic energy?

a. Wavelength.
b. Spectrum.
c. Light intensity.
d. Energy energy.

60. (055) When the number of molecules or ions of absorbing substance present in a solution is decreased, quantity of light absorbed is

a. increased.
b. decreased.
c. not affected.
d. slightly increased.

61. (056) What type of action takes place when substances of interest are measured at a specific wavelength using the spectrophotometer?

a. Substances absorb energy.
b. Substances give off energy.
c. Substances repel light.
d. Substances diffuse light.

62. (057) What two parts of the wavelength selector functions by dispersing the light into its components wavelengths?

a. Prisms and diffraction gratings.
b. Prisms and glass devvices.
c. Diffraction gratings and glass devices.
d. Diffraction gratings and mirror devices.

63. (058) Why does turbidity in a solution change the linear relationship between color and concentration?

a. Light rays are scattered by suspended particles.
b. Light rays are concentrated by suspended particles.
c. Color is absorbed by scatter suspended particles.
d. Color is concentrated by suspended particles.

64. (059) How is optical density related to transmittance?

a. Logarithmically proportional.
b. Inversely proportional.
c. Directly proportional.
d. It is synonymous.

65. (060) How often should the wavelength be checked if a spectrophotometer is in routine daily use?

a. Daily.
b. Weekly.
c. Monthly.
d. Semiannually.

66. (061) What check of the spectrophotometer should be primarily made when the needle is observed to move erratically?

a. Defective controls.
b. Fatigue.
c. Meter repeatability.
d. Grating deterioration.

67. (062) What are the two given primary causes of equipment failures and malfunctions?

a. Poor quality control and operator error.
b. Poor maintenance and defective parts.
c. Poor quality control and defective parts.
d. Poor maintenance and operator error.

68. (062) What given condition would most likely cause erratic readings when aspirating the sample and reading the absorbance using the STASAR III spectrophotometer?

a. Excess sample intake.
b. Insufficient sample intake.
c. Increased sample aspiration.
d. Decreased sample aspiration.

69. (063) What are the two most critical components in the flame photometer?

a. Atomizer and regulator.
b. Monochromator and regulator.
c. Atomizer and flame temperature.
d. Monochromator and flame temperature.

70. (064) What is the principle and purpose of atomic absorption?

a. Measures the absorption of light by unexcited atoms and use to determine a number of metallic elements of low concentration in biologic material.
b. Measures the absorption of light by excited atoms and use to determine a number of metallic elements of high concentration.
c. Measures the absorption of light by excited atoms and use to determine a number of metallic elements of very low concentration.
d. Measures the absorption of light by unexcited atoms and use to determine a number of metallic elements of high concentration.

71. (065) What is the main advantage of fluorometry over absorptive methods of measurement?

a. Increased sensitivity.
b. Increased specificity.
c. Decreased interference from fluorescent compounds.
d. Test not affected by increasing temperature.

72. (066) What action takes place when ion-selective electrodes are dipped into a solution containing a specific ion?

a. The electrodes develop an electrical potential that is a function of the amount of that ion present.
b. The electrodes develop an electrical potential that is greater than the amount of that ion present.
c. The electrodes develop a polarity less than that ion present.
d. The electrodes develop a polarity greater than that ion present.

73. (067) What is the essential part of a continuous flow system?

a. A rotating sample tray.
b. The thermoregulator.
c. A peristaltic pump.
d. The glass coils.

74. (067) To what type of apparatus is the stream of sample directed when deproteinization is required?

a. A dialysis membrane.
b. A thermoregulator.
c. A semicircular groove.
d. A filter lined glass coil.

75. (068) What is difference between the discrete analysis and continuous flow systems of automation?

a. Discrete analysis involves sample treatment and measurement in individual containers; continuous flow analysis employs proportioning reagent and sample measurement.
b. Discrete analysis involves proportioning of reagent and sample measurement; continuous flow employs sample treatment and measurement in individual containers.
c. Continuous flow system involves measurement and treatment of reagents only by proportioning.
d. Continuous flow system involves measurement and treatment of reagents only by sample measurement.

76. (069) What component of the automated instrument is used to read and calculate sample results?

a. Flow-through spectrophotometer.
b. Fluorometer.
c. Recorder.
d. Delay coil.

77. (069) Technicians tend to become complacent and fail to exercise discretion regarding potential problems in the operation of automatic instruments because of what specified reason?

a. The instrument failure minimal.
b. The sound reproducibility of the instrument.
c. The unquestionable accuracy of the instrument.
d. The automated characteristics of the instrument.

78. (070) Which of the following techniques is used in some automated analyzers to eliminate much of the interference caused by turbidity and by hemoglobin or bilirubin in serum samples?

a. Measurement by diffusion.
b. Electrometric measurement.
c. Bichromatic-photmetric measurement.
d. Measurement by reflectance.

79. (071) What has been the major application of computers in the clinical laboratory since 1970?

a. Instrument control.
b. Quality control.
c. Laboratory information.
d. Data control.

80. (071) Which of the following operations is *least* considered to be influenced by computer-control in the newer laboratory instruments?

a. The wavelength scan.
b. The intervals at which observations are made.
c. Visual scan of quality of sample to be used.
d. Data manipulations.

81. (072) Why should a specimen for chemisry tests not be drawn from above the i.v. infusion site?

a. The specimen will be diluted; test results will be erroneous.
b. The specimen will be diluted; test results will all be normal.
c. The specimen is not affected; but test results will be questionable.
d. The specimen is not affected; test results will be normal.

82. (073) Concentrations of constituents in serum or plasma as compared to whole body exhibit a tendency to

a. vary.
b. remain constant.
c. be less stable.
d. be less variable.

83. (074) Which of the following anticoagulants is generally preferred when plasma is required for chemical testing?

a. Lithuin oxalate.
b. Sodium fluoride.
c. Sodium citrate.
d. Heparin.

84. (075) Which of the following conditions should be *least* considered as a cause of hemolysis in drawing and processing blood specimens?

a. Use of too large or small needle.
b. Use of too large or small blood collecting vial.
c. Moisture in a syringe.
d. Vigorous mixing of blood.

85. (076) It has been recommended that you instruct the patient to fast before drawing the sample to ensure which of the following conditions?

a. Laboratory results are compatible with "reference values."
b. Laboratory results are all within the confidence limits.
c. Unexpected laboratory results are observed.
d. Elimination of all unexpected laboratory results.

86. (077) What component or condition present in CSF would be likely to elevate most constituents being measured?

a. Iceteric.
b. White blood cells (cloudy).
c. Serum.
d. Slight hemolysis.

87. (077) Why should you perform a spinal fluid glucose as soon after receiving the specimen as possible, especially if bacteria are present?

a. Pacterial metabolism will decrease sucrose.
b. Bacterial metabolism will increase glucose content.
c. Bacteria utilizing lactose in their metabolism will decrease results.
d. Bacteria utilizing glucose in their metabolism will decrease results.

88. (078) A solution which contains a known and exact composition can *best* be defined as a

a. standard.
b. control.
c. technical grade reagent.
d. reagent grade chemical.

89. (079) Which of the following statements concerning the use of prepared curves is *most* accurate?

a. They may give a false sense of security.
b. Frequent checks are unnecessary if a reliable standard is used.
c. They may be used reliably along with a factor of known derivation.
d. Frequent checks are unnecessary if the control reads within the acceptable range.

90. (080) Which of the following values of glucose determinations *best* represents precision without accuracy if the actual value is known to be 120 mg/dl?

a. 136 mg/dl, 75 mg/dl, 75 mg/dl, 75 mg/dl.
b. 80 mg/dl, 80 mg/dl, 135 mg/dl, 136 mg/dl.
c. 120 mg/dl, 120 mg/dl, 120 mg/dl, 120 mg/dl.
d. 125 mg/dl, 125 mg/dl, 130 mg/dl, 126 mg/dl.

91. (081) Which of the following examples contains a figure that is *not* significant?

a. 1.7890.
b. .17890.
c. .01789.
d. 1.789.

92. (082) Who has the first line of responsbility in the daily quality control program?

a. The technician.
b. The chemistry supervisor.
c. The OIC.
d. The pathologist.

93. (082) Which of the following actions is *most* essential among technicians, supervisors, and the OIC for the success of the clinical chemistry QC program?

a. Frequent attendence to related workshops.
b. Choosing samples for repeat analysis.
c. Providing more written instructions.
d. Frequent communication.

94. (083) What information is *not* required to be included in the daily QC record regarding solutions and instruments in daily use?

a. Solution changes.
b. Instrument repairs.
c. Maintenance procedures.
d. Date reagents completely used.

95. (084) What are the confidence limits of a particular test in mg/dl if $\pm 2.5\sigma$ is the allowable range, $\pm 1\sigma$ is 2, and the mean control value is 100 mg/dl?

a. 95 to 105.
b. 97 to 103.
c. 98 to 102.
d. 99 to 101.

96. (084) The possibility that a control specimen will vary more than $+2.5\ \sigma$ is

a. 32 percent.
b. 12 percent.
c. 4.5 percent.
d. 1.2 percent.

97. (085) What is the standard deviation in 30 conservative determinations of BUN when the sum of the squared differences is 300? (Use table 8-1 or calculator with square root function.)

a. 3.2.
b. 4.2.
c. 5.2.
d. 6.2.

98. (085) What action should be taken when analyzing specific components and repeated results of the normal and abnormal controls are outside of confidence limits?

a. Record results and repeat the entire set of analyses.
b. Check only your performance; you may have omitted a step.
c. Discard that batch of controls, do not record results, but repeat the tests.
d. Discard that batch of controls, record results, but report only the tests.

99. (086) Which of the following statements *best* explains the major purpose of the USAF Graduate Evaluation Program?

a. Determine the long and short term effect of the environment of training.
b. Determine the length of time graduates remain effective in their specialties.
c. Provide feedback data to validate equipment effectiveness.
d. Provide feedback data to validate training effectiveness.

100. (086) To whom are direct correspondence questionnaires sent in the USAF Graduate Evaluation Program?

a. Graduate and OJT supervisor.
b. Students and supervisors.
c. Graduate and supervisor.
d. Students and course supervisors.

END OF EXERCISE

MAIL TO: ECI, GUNTER AFS AL 36118-5643

STUDENT REQUEST FOR ASSISTANCE

PRIVACY ACT STATEMENT

AUTHORITY: 10 USC 8012. PRINCIPAL PURPOSE: To provide student assistance as requested by individual students. ROUTINE USES: This form is shipped with ECI course package, and used by the student, as needed, to place an inquiry with ECI. DISCLOSURE: Voluntary. The information requested on this form is needed for expeditious handling of the student's inquiry. Failure to provide all information would result in slower action or inability to provide assistance to the student.

I.

CORRECTED OR LATEST ENROLLMENT DATA

1. THIS REQUEST CONCERNS COURSE (1-6)	2. TODAY'S DATE	3. ENROLLMENT DATE	4. AUTOVON NUMBER
5. SOCIAL SECURITY NUMBER (7-15) – –	6. GRADE/RANK	7. NAME (First initial, second initial, last name) (17) – (18) – (19-32)	

8. ADDRESS

OJT ENROLLEES: Address of unit training office with zip code.

ALL OTHERS: Current mailing address with zip code.

(33-53)

(54-75)

9. NAME OF BASE OR INSTALLATION IF NOT SHOWN ABOVE

10. TEST CONTROL OFFICE ZIP CODE/SHRED (33-39)

II.

REQUEST FOR MATERIALS, RECORDS, OR SERVICE

X	*Place an 'X' through number in box to left of service requested.*
1	Request address change as indicated in Section I, Block 8.
2	Request Test Control Office change as indicated in Section I, Block 10.
3	Request name change/correction. *(Provide Old or Incorrect data here)*
4	Request Grade/Rank change/correction.
5	Correct SSAN. *(List incorrect SSAN here.)* *(Correct SSAN should be shown in Section I.)*
6	Extend course completion date. *(Justify in "Remarks")*
7	Request enrollment cancellation. *(Justify in "Remarks")*
8	Send VRE answer sheets for Vol(s): 1 2 3 4 5 6 7 8 9 10 Originals were: [ ] Not received [ ] Lost [ ] Misused
9	Send course materials. *(Specify in "Remarks")* [ ] Not received [ ] Lost [ ] Damaged
10	Course exam not yet received. Final VRE submitted for grading on ____________ (date).
11	Results for VRE Vol(s) 1 2 3 4 5 6 7 8 9 10 not yet received. Answer sheet(s) submitted ______________ (date).
12	Results for CE not yet received. Answer sheet submitted to ECI on ____________ (date).
13	Previous inquiry ([ ] ECI Fm 17, [ ] ltr, [ ] msg) sent to ECI on ____________ (date).
14	Give instructional assistance as requested on reverse.
15	Other *(Explain fully in "Remarks")*

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16 G	33	
K	VOL 33-35	GR 36-38
M	33-34	35-40
N	33-35	
P	VOL 33-35	
	TC 36-37	38
	DOE 39-45	
Q	33-34 00	38 1
	MC 39-42	

REMARKS *(Continue on reverse)*

OJT STUDENTS must have their OJT Administrator certify this record.

ALL OTHER STUDENTS may certify their own requests.

I certify that the information on this form is accurate and that this request cannot be answered at this station.

SIGNATURE

ECI FORM DEC 84 17 PREVIOUS EDITION WILL BE USED.

SECTION III: REQUEST FOR INSTRUCTOR ASSISTANCE

NOTE: Questions or comments relating to the accuracy or currency of subject matter should be forwarded directly to preparing agency. For an immediate response to these questions, call or write the course author directly, using the AUTOVON number or address in the preface of each volume. All other inquiries concerning the course should be forwarded to ECI.

VRE Item Questioned:

Course No __________

Volume No __________

VRE Form No ________

VRE Item No ________

Answer You Chose ______ (Letter)

Has VRE Answer Sheet been submitted for grading?

☐ Yes ☐ No

REFERENCE

(Textual reference for the answer I chose can be found as shown below)

In Volume No __________

On Page No __________

In ☐ left ☐ right column

Lines ____ Through _____

MY QUESTION IS:

REMARKS

ADDITIONAL FORMS 17 available from trainers, OJT and Education Offices, and ECI. Course workbooks have a Form 17 printed on the last page.

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MEDICAL LABORATORY TECHNICIAN (CHEMISTRY AND URINALYSIS)

(AFSC 90470)

Volume 2

Laboratory Procedures in Clinical Chemistry
(Part I)

Extension Course Institute
Air University

Acknowledgment

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MCMXLVII

PREPARED BY
SCHOOL OF HEALTH CARE SCIENCES, USAF (ATC)
SHEPPARD AIR FORCE BASE, TEXAS

EXTENSION COURSE INSTITUTE, GUNTER AIR FORCE STATION, ALABAMA

THIS PUBLICATION HAS BEEN REVIEWED AND APPROVED BY COMPETENT PERSONNEL OF THE PREPARING COMMAND IN ACCORDANCE WITH CURRENT DIRECTIVES ON DOCTRINE, POLICY, ESSENTIALITY, PROPRIETY, AND QUALITY.

Preface

GENERAL considerations of the chemical and physical aspects of physiological chemistry were discussed in the first volume of this course. This and subsequent volumes will be more specific in their approach to clinical chemistry. Some discussion is devoted to the analytical aspects of the procedures, with reference to normal values, sources of error, and sample stability. Chapter 1, for instance, discusses electrolytes, blood, gases, and pH. Liver function with its associated tests is covered in Chapter 2, and proteins in Chapter 3. Carbohydrate chemistry, primarily glucose, is developed in Chapter 4. The final chapter is devoted to one of the most difficult analytical areas of the clinical laboratory, enzymes.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to School of Health Care Sciences/MSTW, Sheppard AFB TX 76311. NOTE: Do not use the suggestion program to submit corrections for typographical or other errors.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Behavioral Objective Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFS AL 36118, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 27 hours (9 points).

Material in this volume is technically accurate, adequate, and current as of April 1976.

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NOTE: In this volume, the subject matter is developed by a series of Learning Objectives. Each of these carries a 3-digit number and is in boldface type. Each sets a learning goal for you. The text that follows the objectives gives you the information you need to reach that goal. The exercises following the information give you a check on your achievement. When you complete them, see if your answers match those in the back of this volume. If your response to an exercise is incorrect, review the objective and its text.

Body Metabolism

ANY COMPONENT of metabolism is a metabolite. Included are vitamins, minerals, electrolytes, carbon dioxide, proteins, and water. The term "metabolite" is used arbitrarily and is designed for convenience in discussing body chemistry from a laboratory viewpoint. Electrolytes are given special consideration because of their prominence in laboratory studies and their ironic characteristics. In the first part of this chapter, consideration will be given to anions and cations, which are negatively and positively charged ions, respectively. To sustain life, anions and cations must maintain equilibrium in various parts of the body. This is a dynamic equilibrium, which results in characteristic intracellular and extracellular ion concentrations. In addition to equilibrium concepts, attention is devoted to the physiological and technical asepcts of blood pH measurement. Finally, the direct clinical importance of calcium and phosphorus is explored apart from the discussion of electrolytes. If you know the meaning of a test as it relates to some clinical information, you will find that your interest in your work will increase, which in turn results in careful and accurate analyses.

1-1. Equilibrium of Body Electrolytes

Body electrolytes are considered at some length in this chapter because of their vital role in evaluating patient progress. You will be called upon in almost every major medical and surgical case to render data concerning a patient's electrolytes. With this information the physician regulates and restores the balance of electrolytes which is critical to the well-being of his patient. A variation of 0.5 blood pH can easily cause death. Consider each electrolyte, its normal value, and its physiological significance as you read the first section of this chapter. Also consider how a balance is maintained by the blood buffer systems.

200. Identify terms related to the nature and distribution of electrolytes.

Nature of Electrolytes. A substance that conducts electricity in solution may be classified as an electrolyte. Electrolytes conduct an electric current because they dissociate in solution to produce a significant number of ions. Strong electrolytes are those that are highly dissociated. Sodium chloride furnishes large numbers of sodium and chloride ions in solution and is regarded as a strong electrolyte. As you would expect, a solution of sodium chloride is an excellent conductor of electricity. Weak electrolytes are only slightly dissociated in solution and are poor conductors. Carbohydrates, such as sucrose and glucose, are often presented as examples of nonelectrolytes; they do not dissociate appreciably, and hence do not conduct electricity.

a. Positively charged ions are called *cations* because they migrate to the negative pole or cathode.

b. Negative ions are called *anions* because they migrate to the positive pole or anode when electrodes having an applied potential are immersed in the solution.

The cations most frequently measured in the clinical laboratory are sodium and potassium. The anions most commonly measured are chloride and bicarbonate. The electrolytes present in serum are shown graphically in figure 1-1. Electrolytes function in many ways to sustain the life processes. They are important in maintaining acid-base balance and controlling the pH of the blood within the relatively narrow range from 7.35 to 7.45. They are essential to cell permeability and nerve impulse conductivity, as well as in regulating osmotic pressure. There is practically no limit in describing the importance of electrolytes, and therefore it is more meaningful to discuss individual electrolytes.

In interpreting results, the clinician considers two major aspects normally beyond the scope of the clinical laboratory. The first of these aspects is the relationship between a particular electrolyte and the organ or system involved. For example, the chloride level has a direct relationship to the stomach or pancreas. Second, electrolytes are considered in relationship to each other. For this reason, electrolytes are ordered in groups commonly referred to as electrolyte batteries. Some knowledge of the relationship among

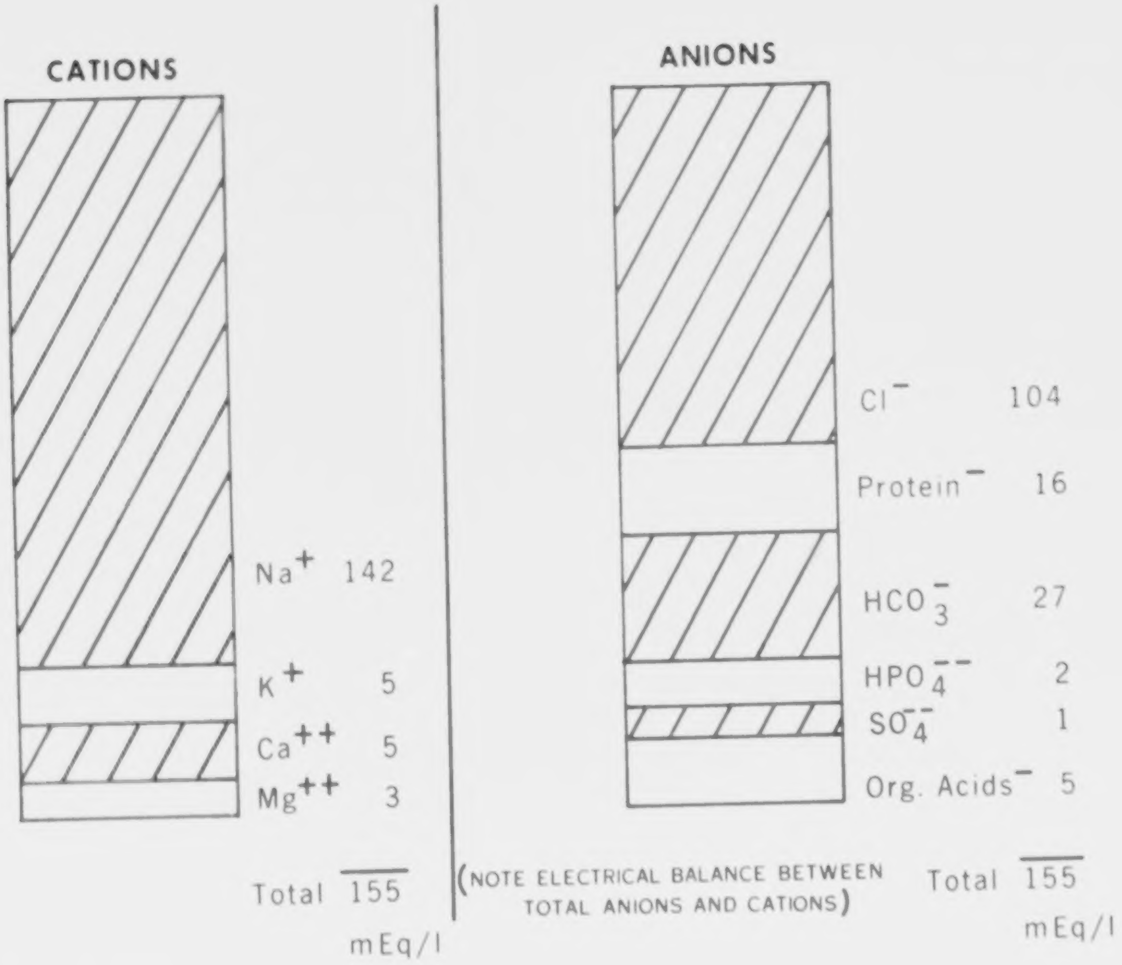

Figure 1-1. Electrolyte composition of serum.

electrolytes is helpful to the technician in achieving results which make sense. In this regard, there is also an element of danger: you should not allow your knowledge (or lack of it) to influence the results you report. In other words, you should not perform a potassium determination with the conscious or unconscious view of arriving at a logical balance with other results. What is logical in the laboratory may not be logical to the physician who is familiar with the clinical condition of the patient.

Distribution of Electrolytes. Electrolytes are not present in equal concentration throughout the body. The various concentrations differ according to whether they are intracellular or extracellular and the kind of tissue involved. This is because cell membranes are selective and only semipermeable. Further, the total of electrical charges within the cell must essentially balance the extracellular charges. Some constituents, such as proteins, do not move freely through cell membranes, but they do possess a charge and are to be considered in ionic equilibrium. The unequal distribution of ions between two areas separated by a semipermeable membrane is established by *Gibbs-Donnan equilibrium*. Without a detailed account of how equilibrium is achieved, we can state that ions shift between the plasma and the cell to maintain electrical neutrality or a slight potential difference. The equilibrium system is composed of both diffusible and nondiffusible components. The dynamic character of cell membranes accounts for various biochemical exchanges between the cell and extracellular fluid. It is understood this is not a membrane in the usual sense, but a selective molecular arrangement which separates the cell from its environment. Because it is more practical to measure extracellular ion levels than ions within the cells, most of your work is with serum or plasma.

Another important aspect in the exchange of materials across a cell membrane is the process of *osmosis.* Osmosis refers to the passage of a solvent through a membrane, more significantly water through a cell membrane. Water passes from a region of lower concentration to an area of higher ion concentration. The resultant physical force exerted in a given area is termed *osmotic pressure.* Keep in mind that osmotic pressure is due to many ions present within and without the cell. Electrolytes commonly measured in the clinical laboratory are a significant part of the ions which maintain functional integrity of the cell. In most, if not all, cases you will be concerned with the fluid surrounding blood cells, through our discussion applies equally to fluid within the blood cells and within other body (somatic) cells.

In addition to assaying serum and plasma, you can secure more valuable data indirectly by measuring the concentration of ions in urine, spinal fluid, and perspiration. Kidneys normally conserve water and electrolytes otherwise lost and are the major means of excreting excesses. Uncompensated loss of water from the body results in dehydration, a condition which may have a profound effect upon ionic concentration. Dehydration occurs in many situations, including sustained febrile conditions, diarrhea, inadequate fluid intake, and many other clinical manifestations. The decrease in fluid volume is primarily extracellular. This is accompanied by a corresponding fall in blood pressure, which may lead to a state of shock. Transfer of water into the cells takes place when the concentration of ions within the cell exceeds extracellular water concentration and is due to the osmotic pressure differential. However, if the loss of water causes the extracellular fluid to become hypertonic, fluid will leave the cells. Both situations occur clinically, the latter being more common.

It is essential for you to realize that electrolyte concentration by itself says nothing of fluid volume, and hence does not provide accurate information about total electrolyte gain or loss. Sodium and potassium may be lost from the body along with large quantities of water without any appreciable effect on electrolyte concentration, since concentration depends upon both amount of solute and volume of solvent, in this case blood volume. In most cases, the clinician is able to deduce the true extent of the deficit from both laboratory and clinical data, which emphasizes the need to interpret laboratory results in context.

Exercises (200):

Match each term in column B with the correct definition or description in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

___ 1. Cations most frequently measured.
___ 2. Anions most commonly measured.
___ 3. The unequal distribution of ions between two areas separated by a semipermeable membrane.
___ 4. Resultant physical force exerted when water passes from a region of lower concentration to an area of higher concentration through cell membrane.
___ 5. Due to many ions within and without the cell.
___ 6. Conserves water and electrolytes otherwise lost.
___ 7. Uncompensated loss of water from the body.
___ 8. Can severely affect ionic concentration.
___ 9. Must essentially balance the extracellular charges.
___ 10. Possess electrical charges and are considered to be in ionic equilibrium.

Column B

a. Carbohydrates.
b. Sodium and potassium.
c. Osmotic pressure.
d. Protein.
e. Kidney.
f. Chloride and bicarbonate.
g. The total of electrical charges within the cell.
h. Bladder.
i. Gibbs-Donnan equilibrium.
j. Dehydration.

201. Indicate the relationship of sodium, potassium, and chloride to bodily functions and the effects of abnormal levels.

Sodium. The concentration of sodium in plasma exceeds the concentration of any other cation. Actually, sodium comprises more than 90 percent of all the cations. The normal range is 138 to 146 mEq/L. The daily dietary intake of sodium is usually substantial. That which is not needed is excreted in the urine and to a lesser extent in perspiration. Most of the body sodium is present in fluids, but large amounts are found in the skeletal system. It is more common to find a decrease than to find an increase in serum sodium. As stated previously, there may be a loss of sodium with proportional loss of fluid, thus causing little change in the concentration of sodium. Conversely, water retention can hide the fact that total body sodium may have increased. Both sodium and potassium are measured with the flame photometer and reported in milliequivalent terms. Sodium concentration is often evaluated with respect to the concentration of bicarbonate and chloride ions. Sodium has a primary function in osmotic equilibrium, cell permeability, and muscle irritability.

Potassium. The normal serum potassium level in the body is 3.8 to 5.0 mEq/L. However, most of the potassium is present within body cells, with some in the extracellular fluid. It is for this reason that you should not perform potassium determinations on hemolyzed serum. The result would inevitably be higher whenever serum is hemolyzed. Elevated plasma potassium (hyperkalemia) is associated with disease of the heart and central nervous system. Regulating potassium levels is a problem for the physician in renal failure, Addison's disease, and dehydration. A low serum potassium is frequently a postoperative problem because the patient most likely has received fluids low in potassium. The physician frequently orders postoperative potassium levels. Low serum potassium levels (hypokalemia) may also occur in various other conditions, especially diarrhea. Potassium deficiency results in cell damage, particularly cells of the kidney tubules. We frequently associate potassium with muscle activity, nerve impulses, and, of course, acid-base balance. Like sodium, potassium functions in maintaining osmotic balance between the cells and extracellular fluid.

Chloride. The chloride content of plasma is not equal to the sodium concentration. This is best explained in terms of the Gibbs-Donnan equilibrium previously mentioned. In the presence of negatively charged protein, there must neccessarily be more sodium ions and fewer chloride ions to maintain electrical neutrality. In more general terms, in the presence of a nondiffusible protein anion, the concentration of the diffusible sodium cation would be higher than that of the diffusible chloride anion. It should also be kept in mind that total ionic concentration within the cell is not identical with the extracellular ionic concentration, and as a result, there is a difference is osmotic pressure.

As you know, chloride is usually reported based on the milliequivalent weight of sodium chloride. It would appear that little justification exists for expressing the chloride concentration in terms of sodium chloride, since the sodium concentration of plasma does not equal chloride concentration. In reality, the report to the physician reflects the same relative situation whether chloride content is reported in terms of the milliequivalent weight of chloride or of sodium chloride.

It may sometimes be difficult for you to think in terms of ions rather than compounds, and you should realize that some current laboratory practice is a carryover from days of less enlightened theory. You can easily recognize how chloride came to be reported as NaCl because sodium and chloride ions are closely associated. Most chloride taken into the body is in the form of sodium chloride, and a deficiency of one is likely accompanied by a deficiency of the other. A decreased plasma chloride develops in excessive perspiration and conditions accompanied by a decrease in plasma sodium.

Any interference with the reabsorption of the intestinal secretions will cause alterations in the fluid and electrolyte balance. The most frequent disturbances of gastrointestinal function are vomiting and diarrhea. While gastric juice has a relativley low sodium content, there is a high concentration in the gastric mucus. Irritative conditions which cause vomiting will also induce increase in the production of mucus so that considerable amounts of sodium may be lost.

Situations do occur in which cloride deficiency is more striking than loss of sodium. Malabsorption of gastric juice or diminished production can be the likely causes. When chloride loss is greater than sodium loss, there will be an elevation of the serum bicarbonate to maintain electrolyte balance. The basic condition which then results is hypochloremic alkalosis. When sodium loss is greater than chloride loss, as in obstruction of the lower portion of the small intestine, there is a decrease in serum bicarbonate which results in acidosis.

Exercises (201):

1. Most of the sodium is present in which constituent of the body?

2. What factor can hide a loss of sodium? An increase of sodium?

3. What threefold function does sodium serve in the body?

4. In what constituent of the body is most of the potassium present?

5. Why is it not recommended that potassium determinations be performed on hemolyzed serum?

6. What conditions are associated with elevated plasma potassium (hyperkalemia)?

7. What conditions are associated with lowered plasma potassium (hypokalemia)?

8. Relating briefly to the Gibbs-Donnan equilibrium, what condition must exist in the presence of charged protein in order to maintain electrical neutrality?

9. What situations may occur in which chloride deficiency is more pronounced than the loss of sodium?

10. When the chloride loss is greater than sodium loss, what condition results to maintain electrolyte balance?

11. When sodium loss is greater than chloride loss, what specific condition may result?

202. State the purpose, procedures, and reasoning behind chloride determinations.

Determination of Chloride. There are a variety of titrimetric methods to determine the concentration of chloride ions in body fluids. They are commonly performed on serum, spinal fluid, perspiration, and sometimes on urine. The procedure most widely accepted in Air Force facilities is that of Schales and Schales. A sample is titrated with a solution of mercuric nitrate. In the presence of S-diphenylcarbazone, after all mercuric ions have united with the chloride ions to form ionized mercuric chloride, the excess mercuric ions will produce a lavender color. If serum is titrated directly without preparation of a PFF, the pH is sometimes high enough to produce a purple color immediately upon addition of mercuric nitrate. To prevent confusion, you may add a drop or two of 0.1N HNO_3 or continue to add mercuric nitrate quantitatively until the color disappears. As discussed in Volume 1 of this CDC, we recommend that you prepare a protein-free filtrate to prevent binding of mercuric ions with the protein. The normal value for spinal fluid chloride is 123 to 128 mEq/L, notably higher than the normal for serum. For reasons of consistency with other electrolyte values, chloride is not usually reported in mg-%.

Another method of measuring chloride makes use of silver compounds. The technique of Volhard, introduced just before the turn of the century, uses silver nitrate. After precipitation, the excess silver ions are titrated with a thiocyanate. A more recent procedure using silver compounds is that of Sendroy, which was introduced in 1937. In the Sendroy procedure, the chloride ion is reacted with silver iodate ($AgIO_3$), resulting in the formation of insoluble silver chloride and iodate ions. After the solution is filtered or centrifuged to remove AgCl, the iodate ion concentration is determined as an index of chloride present before the reaction. The iodate ions are measured in various ways, usually by a thiosulfate-starch titration. This procedure can be used with accuracy, but is not quite as simple as the Schales and Schales method.

Chloridometer. This is a very convenient instrument for the determination of chloride. The chloride in solution is titrated with silver ions, which are formed at a constant rate by the passage of an electric current. By comparing the time required to titrate samples and known standards, the concentration in the former can be readily calculated. The newer models have direct readout, so that the instrument can be made to read exactly 100 mEq/L when a standard of that concentration is titrated.

Pilocarpine iontophoresis. Sweat electrolyte determinations are essential for the early diagnosis and treatment of children with cystic fibrosis. This disease is one of the most serious chronic diseases of childhood. Sweat chloride is most often used as a screening test since in cystic fibrosis, these chloride values range above 60 mEq/L, while in normal children the concentration does not exceed 60 mEq/L. Heterozygous individuals are reported to range from 40 to 60 mEq/L in sweat chloride concentration. Porous paper and agar, impregnated with reactant chemicals, have been used as a simplified sweat chloride screening test. The reliability of many screening procedures is frequently questioned.

The problem of obtaining a sufficient quantity of sweat, without undue stress to the child, has been solved with the introduction of pilocarpine iontophoresis to induce localized sweating. Many iontophoresis instruments are available for this procedure. A slight electrical charge from the iontophoresis instruemnt causes pilocarpine to enter the skin tissue to stimulate locally increased sweat gland secretion. The area (forearm or leg) is thoroughly washed and rinsed before chemically clean gauze or porous paper is placed to absorb the sweat. Ultramicro titrimetric (Schales and Schales) or electrometric determinations for chloride have been used directly on sweat obtained by centrifugation. Usually, a sufficient amount is recovered to also determine the sodium concentration on a diluted specimen.

Exercises (202):

1. Why is a drop of nitric acid sometimes added to the serum before a direct serum chloride titration by the Schales method?

2. Why are the chloride values not in mg-%?

3. In the technique of Volhard for chloride determinations, which uses silver nitration, what procedure is accomplished after precipitation?

4. How is chloride measured using the chloridometer?

5. For what purpose is the sweat electrolyte determination performed?

6. What is the purpose of the pilocarpine in the pilocarpine iontophoresis?

203. State the purpose, identify terms, and cite deficiency conditions in blood gas and pH determinations.

Bicarbonate, Carbon Dioxide Content (Total CO_2), Carbonic Acid, pCO_2, and pH. No electrolyte battery is complete without a determination of carbon dioxide content. Some instruments measure carbon dioxide volumetrically; others may be manometric, as shown in figure 1-2. Subsequent discussion will show you the difference in interpretation of CO_2 content and CO_2 combining power. These manometric and volumetric procedures, which have been used extensively in hospital laboratories, are published in detail in AFM 160-49, *Clinical Laboratory Procedures—Chemistry and Urinalysis.* More recently, an automated colorimetric method employing earlier titrimetric principles has been introduced for the Autoanalyzer.

The fundamental purpose of measuring CO_2 content is to enable the physician to evaluate buffering capacity of the blood to maintain a pH of 7.4. Relatively little carbon dioxide is normally present in the plasma as dissolved CO_2. Instead, it is carried in the cells and plasms in combination with hemoglobin and other proteins in the form of bicarbonate, and a small amount as carbonic acid. To maintain a pH of 7.4, a ratio of 20:1 must be maintained between bicarbonate and carbonic acid. (There are also other buffer systems in the body that are not discussed here.) Before studying the Henderson-Hasselbalch equation, you should review the concept of ionization in Volume 1 and that of pH in Volume 1. Now, let us define the following terms related to bicarbonate and carbon dioxide content:

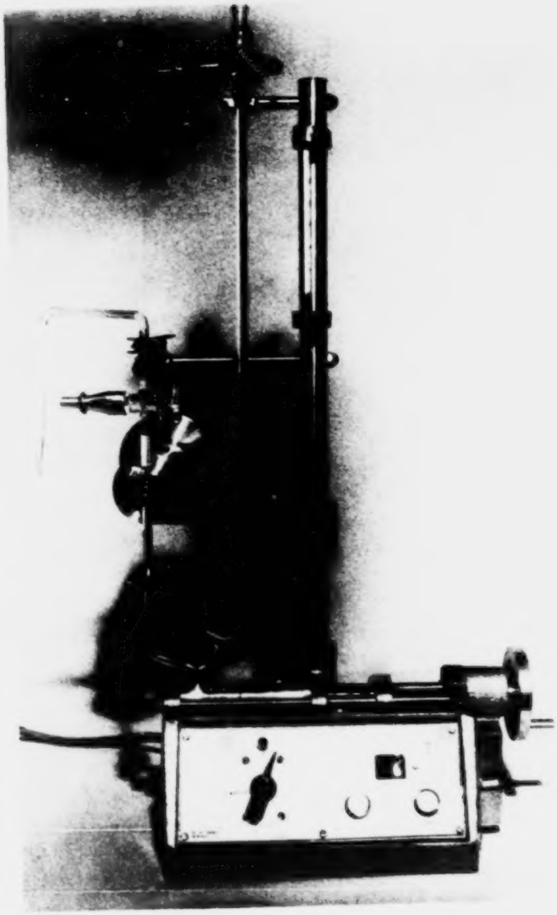

Figure 1-2. Natelson microgasometer for the manometric determination of carbon dioxide.

Bicarbonate. The bicarbonate (HCO_3) is the second largest fraction of the anions in plasma. The ionized bicarbonate (HCO_3^-) and the carbonate (CO_3^{--}) are customarily included in this fraction, as well as the carbamino compounds.

Carbonic acid. This fraction of blood plasma or serum includes the undissociated carbonic acid ($HHCO_3$) and the physically dissolved (anhydrous) CO_2.

Total CO_2. The total CO_2 content of blood, plasma, or serum consists of an ionized fraction that contains HCO_3^- (and CO_3^- compounds) and an un-ionized fraction that contains $HHCO_3$ and physically dissolved (anhydrous) CO_2:

$$[\text{Total } CO_2] = [HCO_3^-] + [HHCO_3]^*$$

CO_2 combining power. The value of the CO_2 combining power is an index of the amount of CO_2 that

can be bound by serum, plasma, or whole blood as HCO_3^- at a pCO_2 of 40 mm at 25° C.

pCO_2. The pressure of a mixed gas, such as air, is the sum of the partial pressures of the individual gases. That part of the pressure which is contributed by CO_2 is called the partial pressure of CO_2 (pCO_2). It is usually expressed in millimeters of Hg. The only place in the body where the blood is in contact with a gas phase is in the lung alveoli.

pH. The pH is the negative logarithm of the hydrogen ion concentration ($pH = -\log [H^+]$). Thus, the average pH of blood (7.40) corresponds to a hydrogen ion concentration of 0.000 000 04 (4×10^{-8} moles/L), or the H^+ is $10^{-7.4}$ moles/L. It can reasily be seen that the pH value is a more convenient figure than the hydrogen ion concentration; this was one reason for introducing the concept of pH.

You will remember that pH may be expressed as a negative logarithm of the hydrogen ion concentration, $pH = -\log [H^+]$. At a pH of 7.4, then, the $[H^+]$ is $10^{-7.4}$ moles per liter. The equilibrium expression for ionization of any weak acid, such as H_2CO_3, in aqueous solution was detailed in Volume 1. This ionization expression for carbonic acid is

$$H_2CO_3 = H^+ + HCO_3^-$$

where H_2CO_3 is the acid, H^+ the hydrogen ions; and HCO_3^- the bicarbonate ions. The ionization constant (K_a) is the ratio of the product of hydrogen and bicarbonate ion concentrations to the concentration of carbonic acid, or mathematically

$$K_a = \frac{[H^+][HCO_3^-]}{[H_2CO_3]}$$

It is more convenient to express this in its logarithmic form ($-\log K_a$) when making calculations with pH. In the formula, $pH = -\log [H^+] = 10^{-pH}$; therefore, $pK_a = -\log K_a = 10^{-K_a}$. The pK_a for this reaction in blood is dependent upon temperature, ionic strength, and the presence of protein.

Exercises (203):

1. What is the purpose of measuring the CO_2 content?

2. In order to maintain a pH of 7.4, what ratio must be maintained between the bicarbonate and carbonic acid?

3. What two fractions are customarily included in the bicarbonate?

4. Which fraction of blood plasma or serum includes the undissociated carbonic acid and the physically dissolved (anhydrous) CO_2?

5. What are the constituents given for the total CO_2 contents of blood, plasma, or serum?

6. Of what value is the CO_2 combining power?

7. What does "the pCO_2" mean?

8. What does the symbol $[H^+]$ mean?

9. How is the symbol for hydrogen, H, different from the symbol $[H^+]$?

10. What is known as the ionization constant?

204. Indicate whether given statements correctly reflect the relationship of the Henderson-Hasselbalch equation to blood buffers in maintaining the acid-base equilibrium.

Henderson-Hasselbalch Equation. In terms of the Henderson-Hasselbalch equation for buffers, the carbon dioxide buffering system is expressed as follows:

$$pH = pK_1 + \log \frac{[HCO_3^-]}{[H_2CO_3]} \qquad (1)$$

The pK value for this buffer systemsis 6.1. Using this value, we could write the equation as follows:

$$10^{-7.4} = 10^{-6.1} \frac{[HCO_3^-]}{[H_2CO_3]}$$

Then solve for the ratio as follows (note the antilog of 1.3 is 20):

$$\frac{[HCO_3^-]}{[H_2CO_3]} = \frac{10^{-7.4}}{10^{-6.1}} = 10^{-1.3} = \frac{1}{20}$$

You will find that the pK value in (1) above is not the pK of carbonic acid. Rather, it is the pK for dissolved CO_2, which is proportional to carbonic acid concentration.

The partial pressure (pCO_2) of carbon dioxide in the lungs will determine the amount of dissolved CO_2 and the small amount of carbonic acid that will be in equilibrium in the plasma. This relationship is expressed mathematically as

$$\begin{array}{l} H_2CO_3\ mM/L \\ \qquad \text{or} \quad = a.pCO_2 \\ CO_2\ \text{dissolved} \end{array} \tag{2}$$

where "a" is a constant known to be 0.03 for dissovled CO_2 in mM/L. It follows that pCO_2 is proportional to carbonic acid or dissolved CO_2 concentration.

Carbon dioxide will diffuse to any other phase (solid, liquid, or gas) which has a lower partial pressure and is exposed to the plasma. This is the reason why blood specimens for CO_2 content and direct pH measurements *must be drawn anaerobically*. This can be done in a sealed vacuum tube system or capillary tube if the container is filled completely to minimize a gas (air) phase in contact with the blood specimen. In the past, blood was collected anaerobically under oil, but this method has not been used generally since the advent of suitable vacuum tubes. Heparin is the anticoagulant of choice for this collection. The equation in (2) may be substituted in the Henderson-Hasselbalch equation (1). Thus:

$$pH = pK_1 + \log \frac{HCO_3^-}{a \cdot pCO_2} \tag{3}$$

CO_2 content (total CO_2) includes both bicarbonate and CO_2 dissolved (carbonic acid, H_2CO_3),

$$CO_2\ \text{content} = HCO_3^- + CO_2\ \text{dissolved}$$

Substituting equation (2) for carbon dioxide dissolved:

$$CO_2\ \text{content} = HCO_3^- + (a.pCO_2)$$

and

$$HCO_3^- = CO_2\ \text{content} - (a.pCO_2)$$

Substituting this in the Henderson-Hasselbalch equation (1),

$$pH = pK_1 + \log \frac{[CO_2\ \text{content} - (a.pCO_2)]}{a.pCO_2} \tag{4}$$

This formula describes the relationship of carbon dioxide partial pressure (pCO_2) to acid-base balance as it is expressed in the Henderson-Hasselbalch equation. The significance becomes more apparent when you realize that this acid-base relationship can be defined if either the pH or CO_2 content is determined at a known pCO2.

Astrup Approach to Acid-Base Metabolism. This concept is the basis for a relatively new approach to quantitation of the factors involved in acid-base balance as proposed by one authority, Astrup, in 1961. The Astrup approach uses a capillary glass electrode to measure blood pH at two known pCO_2 values, one high and one low. Whole blood is equilibrated with each of these gases at 38° C before the pH is measured. These two points are plotted on a prepared nomogram with pH versus pCO_2, as shown by points A and B in figure 1-3. When these two points are joined by a straight line, all relevant acid-base data can be obtained from the nomogram. When the pH of an anaerobic blood specimen is determined, the actual pCO_2 is obtained from the point of intersection with the straight line plotted previously. This is indicated by point F and the dotted lines in figure 1-3. The actual pCO_2 and pH values of the patient may now be substituted in the Henderson-Hasselbalch equation (4) to establish the state of acid-base equilibrium.

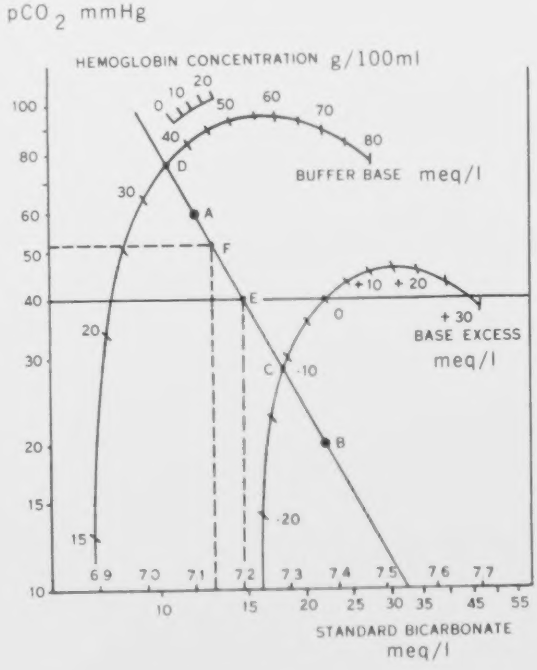

Figure 1-3. Astrup nomogram for calculation of the relevant acid-base values of a blood sample.

Exercises (204):

Indicate whether the following statements are true (T) or false (F) and correct those that are false.

T F 1. The pK value of the Henderson-Hasselbalch equation for buffers is 7.4.

T F 2. The partial pressure (pCO_2) of carbon dioxide in the lungs will determine the amount of dissolved CO_2 and the small amount of carbonic acid that will be in equilibrium in the plasma.

T F 3. In the Henderson-Hasselbalch equation, pCO_2 is proportioned to carbonic acid and dissolved CO_2.

T F 4. Carbon dioxide will diffuse to any other phase (solid, liquid, or gas) which has a higher partial pressure and is exposed to the plasma.

T F 5. Sodium citrate is the anticoagulant of choice for collection of blood gases.

T F 6. CO_2 content (total CO_2) includes both the bicarbonate (HCO_3) and dissolved carbonic acid (H_2CO_3).

T F 7. The acid-base relationship can be defined if either the pH or CO_2 content is determined at a known pCO_2.

T F 8. The Astrup approach used a capillary glass electrode to measure blood pH at three known pCO_2 values—high, moderate, low.

T F 9. Whole blood is equilibrated with each of the gases at 38° C before the pH is measured.

205. Identify terms associated with acid-base determination, the buffering capacity of the blood, and conditions which occur due to acid-base imbalance.

Terms for Acid-Base Determinations. Astrup introduced several new terms for determinations which may be calculated from his nomogram. They are defined as:

Standard bicarbonate—the bicarbonate concentration of plasma from completely oxygenated blood equilibrated at 38° C and a pCO_2 of 40-mm Hg.

Base excess—the excess (positive value) or deficit (negative value) of base in mEq/L, assuming normal base excess is zero at pH 7.38, and a pCO_2 of 40-mm Hg on completely oxygenated blood.

Buffer base—the sum of all buffer anions (mEq/L) in 1 liter of blood. The arrangement of these values can be seen on the nomogram in figure 1-3. Several American-made pCO_2 instruments are available with a direct readout of pCO_2 and nomograms for other calculations in acid-base balance.

Significance of acid-base balance to laboratory technician. Let us summarize what the previous discussion of acid-base balance means to you as a laboratory technician. First, it is obvious that since bicarbonate represents two-thirds of the blood-buffering capacity, an adequate measurement of bicarbonate will establish the state of acid-base balance. However, the bicarbonate determination must be made with regard to clinical physiology and the Henderson-Hasselbalch equation. This equation (1) has three unknowns: pH, bicarbonate, and carbonic acid concentrations. The equation can be solved if any two of these unknowns are determined. However, this form of the equation is valid only when the pCO_2 is normal; that is, when there is no pulmonary involvement or abnormal respiration. The substituted form of the Henderson-Hasselbalch equation (4) accounts for any change in pCO_2 and has three unknowns: pH, CO_2 content, and pCO_2. It is evident that either pCO_2 or pH must be determined in addition to CO_2 content to adequately solve this equation.

Buffering Capacity. Although most of the buffered carbon dioxide is transported in the plasma as bicarbonate, more than three-fourths of the total carbon dioxide is carried by the red blood cells and the plasma. As bicarbonate (HCO_3^-) moves from the red blood cells into the plasma, chloride ions move into the cells to maintain electrical neutrality. You may recall that there are primarily potassium ions within the cells and sodium ions in the plasma to balance the anions present. The movement of chloride to replace bicarbonate inbalancing potassium is referred to as the *chloride shift*. The entire problem is one of preserving the blood pH while the patient may encounter either respiratory or metabolic activity that tends to change the ratio of carbonic acid to bicarbonate.

The various conditions that may occur are respiratory acideosis or alkalosis and metabolic acidosis or alkalosis. Each condition characteristically affects the ratio and may be compensated by various factors. In metabolic acidosis there is a decrease in bicarbonate. This may occur in renal disease or in diabetes mellitus. Diabetic acidosis results from loss of bicarbonate and increase of carbonic acid following the release of hydrogen ions from the breakdown of fats and proteins (gluconeogenesis). The opposite is encountered in metabolic alkalosis, wherein there is little or no change in carbonic acid content of the blood. This may occur as a result of intestinal obstruction, in which case the chloride may also decrease. In certain respiratory diseases there is an increase in carbonic acid relative to bicarbonate, and the effect is respiratory acidosis. Finally, in respiratory alkalosis, the carbonic acid decreases with a disproportionate decrease in the bicarbonate. The chloride may be elevated as well as in hyperventilation. Knowledge of the plasma CO_2 content and pCO_2 or pH aids the clinician immeasurably in evaluating symptoms relating to the treatment of his patient.

Exercises (205):

Match the following terms and conditions of column B with the statement in column A to which they most nearly relate.

Column A

___ 1. Deficit (negative value) of base in mEq L., assuming normal base excess is zero at pH 7.38, and a pCO_2 of 40-mm Hg on completely oxygenated blood.
___ 2. The sum of all buffer anions in 1 liter of blood.
___ 3. pH, bicarbonate, and carbonic acid concentrations.
___ 4. Must be determined in addition to CO_2 to solve the Henderson-Hasselbalch equation.
___ 5. The shift which takes place between the plasma and the cell with respect to chloride and bicarbonate to maintain electrical neutrality.
___ 6. Condition in which there is a decrease in bicarbonate.
___ 7. Results from loss of bicarbonate and increase of carbonic acid following the release of hydrogen ions from the breakdown of fats and protein.

Column B

a. Unknowns of Henderson-Hasselbalch equation.
b. Base excess.
c. pCO_2 and pH.
d. Buffer base.
e. Metabolic acidosis.
f. Chloride shift.
g. Respiratory acidosis.
h. Metabolic alkalosis.
i. Diabetic acidosis.
j. Respiratory alkalosis.
k. A patient loses CO_2 by hyperventilation.

Column A

___ 8. May occur as a result of intestinal obstruction, wherein there is little or no change in carbonic acid content and the chloride may decrease.
___ 9. An increase in carbonic acid relative to bicarbonate.
___ 10. The carbonic acid decreases with a disproportionate decrease in bicarbonate.
___ 11. An increase in chloride and a decrease in bicarbonate.

206. Indicate the method for blood pH analysis by citing types of electrodes used, reasoning behind, and factors that affect the determination.

Electrometric Measurement of pH. The physician does not always have a clinical history or prior knowledge of a patient's disorder. Lacking information on the etiology of a patient's condition, the physician does not necessarily know if the diagnosis should be "acidosis" or "alkalosis." Further, this cannot be determined with certainty from carbon dioxide levels alone. It is, therefore, necessary to measure blood pH directly or to calculate pCO_2 in addition to measuring CO_2 content. Because of the narrow limits within which blood pH must be measured, only specially designed pH meters are sensitive enough for this purpose. Even in diabetic coma, the pH of the patient's blood may not vary from the normal by more than 0.5 pH unit. There are pH meters available today which can measure to ± 0.001 pH unit on samples as small as 0.015 ml.

Regardless of design or sensitivity, pH meters all measure electromotive forces. This cannot be accomplished with a singel electrode, because potential difference exists between two electrodes. With one of the electrodes as a reference electrode, the potential of the other is measured in comparison with this standard. Various electrodes are in use; four are discussed here. The first is a hydrogen electrode, which is arbitrarily assigned a value of 0.0 volts. It is composed of a strip of platinum which has been coated with platinum black. When this electrode comes in contact with hydrogen gas and water, electrons are released and adhere to the metal. The electrode will then have a negative charge. Hydrogen electrodes are prepared in which the hydrogen gas is under a pressure of one atmosphere. If the temperature is held constant, the electrode will maintain a standard value. This type of electrode is *not* used for blood because the carbon dioxide would be removed by the hydrogen. The hydrogen electrode is the standard electrode upon which all others are based.

A second type of electrode is the calomel electrode. This electrode is usually the reference electrode used in blood pH measurements. Calomel electrodes are less difficult to prepare and maintain than hydrogen electrodes. They are composed of mercury, a paste of Hg_2Cl_2, and a solution of patassium cloride (KCl). Under standard conditions a saturated calomel electrode will have a potential of 0.256 volt.

A third type of electrode used is the quinhydrone electrode. It is used in solutions in which hydrogen of the hydrogen electrode would react with a component of the solution. The rationale behind this type of electrode is that hydroquinone ($C_6H_4(OH)_2$) reacts with water to yeild quinone ($C_6H_4O_2$) and hydronium ions with the release of electrons.

A fourth type of electrode is the glass electrode which consists of a metal such as platinum immersed in an acid solution and inclosed in permeable glass. This is the blood pH electrode. The principle of the glass electrode is quite involved, but essential characteristics are established by a difference in hydronium ion concentration on each side of a glass membrane. For this reason, most of the older glass electrodes on the market are not valid for solutions of high pH or very low pH, since what passes through the glass membrane is affected by extremes in pH.

Regardless of the type of electrode, pH meters measure potential difference between two electrodes, one of which is standardized. Temperature affects the determination and must be controlled whenever pH is measured within narrow limits. Potential of the circuit established is measured with a potentiometer after amplification. Only when the cell resistance exceeds 1 megohm is a special voltmeter necessary. This is the case when glass electrodes are a part of the system. Circuit diagrams for particular pH meters can be obtained from the manufacturer of the particular instrument. It is sufficient for you, as a technician, to understand how it is possible to determine hydrogen ion concentration electrically. Models vary widely in mechanical design and in the adjustments they require. Some are equipped with thermocompensators which eliminate the necessity for manual temperature adjustments.

Standardization is always necessary, and is accomplished with one or more known buffers. The most common problem in standardizing a pH meter arises when you do not know the correct pH value of your standard buffer. A series of buffers should be kept on hand, some of which should be procured from a commercial source. Also, you should give special attention to conditions under which the buffers are stored. Contamination is always a threat to reliability, especially when several people have access to standard buffers. Finally, a pH meter should be used to measure the pH of solutions for which the instrument and electrodes were designed.

As mentioned earlier, all instruments are not suitable for the measurement of blood pH. A blood pH meter, as shown in figure 1-4, is a highly specialized instrument. Blood pH, pCO_2, and pO_2 determinations may be performed by using the Corning Model 165 pH Blood Gas Analyzer on a single whole blood sample. O_2 measurement is performed by a Clark type electrode. A Severinghayse-Stow type electrode measures CO_2, and a flow through glass capillary and refernece assembly measures pH. The manufacturer's setup and operating instructions should be consulted prior to performance of these tests.

Exercises (206):

1. Why is it necessary to measure blood pH or to calculate pCO_2 in addition to measuring CO_2 content?

2. Why is it necessary to have only specially designed sensitive pH meters for measurement of blood pH?

3. What is the standard electrode which serves as the arbitrary basic for electromeasurement of pH?

4. Why is it important to know the type of electrode on a particular instrument?

5. Regardless of design or sensitivity, what specifically do pH meters measure?

6. Why is the standard electrode not used for blood pH measurements?

7. Of what is a calomel electrode composed?

8. What type of electrode is usually used in blood pH measurements?

9. What factor affects the pH determination and must be controlled whenever pH is measured within narrow limits?

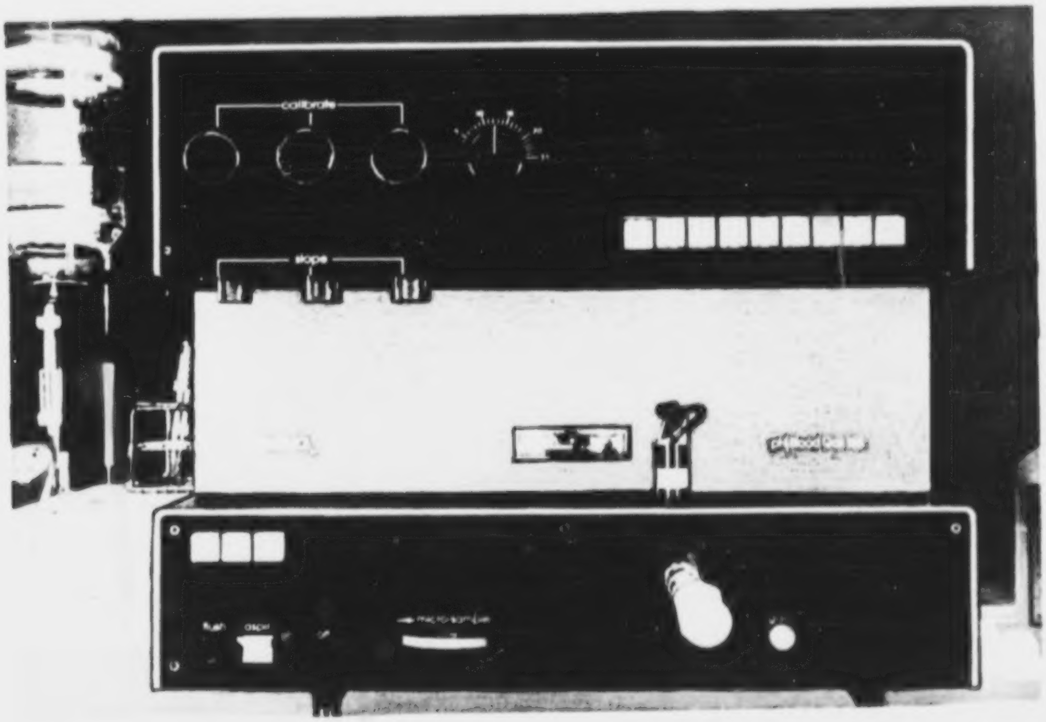

Figure 1-4. Model 165 pH blood gas analyzer.

10. What is the main threat to the reliability of standard buffers?

1-2. Other Inorganic Metabolites

Two very commonly assayed inorganic ions are calcium and phosphorus. They are frequently requested in the clinical evaluation of metabolic disorders. As you read, try to appreciate the relationship between calcium and phosphorus levels. In addition, magnesium and lithium are more frequently assayed through the widespread use of atomic absorption spectroscopy.

207. Identify the method, principle, reagents, and conditions for calcium determination.

Calcium. The most abundant cation of the body is calcium because it makes up a large part of the skeletal system. Calcium is also present in nerve and muscle tissue as well as the coagulation mechanism of blood. It may be recalled that many of the anticoagulants added to blood specimens function by chelation of calcium. One does not normally encounter a calcium deficiency which is severe enough to interefere with the body's coagulation mechanism.

Normal levels. The normal intake of calcium amounts to several grams per day, with an essential daily requirement of just over 1.5 grams. Various factors facilitate the absorption of calcium, including pH of the intestine and the presence of Vitamin D. The normal serum level is 4.5 to 5.5 mEq/L or 9 to 11 mg-%, with no reported variation related to diet or fasting condition of the patient. Blood cells, themselves, contain a negligible amount of calcium. Besides being in the form of ions, calcium in the nondiffusible form combines with certain plasma constituents. Clinically, a decrease in ionizable calcium is associated with tetany, a condition characterized by spasms of the extremities. Tetany may occur during and following pregnancy, especially during lactation, because of increased demand for calcium.

There are other causes for decreased calcium, including removal or deficiency of the parathyroid glands, alkalosis and Vitamin D deficiency. The influence of the parahyvroid glands is profound. In hyperparathyroidism, serum calcium may be elevated to 20 mg-% or more. The level of calcium is invariably closely associated with phosphorus levels of the serum, and these two elements tend to maintain a constant ratio. There is usually a concomitant increase in serum phosphate levels as there is a decrease in calcium values. However, there may be deficiency of both calcium and phosphorus, as in rickets.

Determining calcium levels. There are many techniques for determining serum calcium levels. In some methods calcium is precipitated with some anion like oxalate, with quantitative estimation of the anion. Other methods titrate directly, using a suitable indicator, such as ammonium purpurate. A number of serum constituents interfere with direction titration and, in addition, end points are not usually clear enough to establish this as a method of choice. For example, EDTA interferes with the chemical determination of calcium by titration or colorimetric methods and may interfere with oxalate precipitation, but it will not interfere with the determination by flame photometry or atomis absorption.

It is not possible to discuss in detail all of the various techniques available for the assay of calcium. Perhaps the most widely accepted manual chemical method in US Air Force facilities is an adaptation of the Ferro and Ham method. In this method, calcium is precipitated as the insoluble salt of chloranilic acid. It is washed free of excess chloranilic acid, using isopropyl alcohol. The precipitate of calcium chloranilate is then dissolved in a solution of ethylenediaminetetraacetic acid (tetrasodium salt). The resulting pink solution is compared photometrically against a similarly prepared standard. Where urine is used as the specimen, either the flame photometer method or the Sulkowitch determination is normally used. In the Sulkowitch test, calcium is precipitated with oxalic acid reagent and the degree of turbidity is noted. Phosphates do not precipitate with calcium, but the degree of turbidity may be affected by other urinary constituents. There is some question as to the value of such an approximation, and it is generally interpreted rather liberally.

Exercises (207):

Match the following by placing the letter of the column B item beside the column A item or items that most nearly describe it. Each item in column B may be used once, more than once, or not at all.

Column A	*Column B*
___ 1. The most abundant cation of the body.	a. Phosphorus (phosphate). b. Calcium.
___ 2. Facilitate the absorption of calcium.	c. Intestinal pH and vitamin D. d. Removal or deficiency of parathyroid glands and alkalosis.
___ 3. Normal values 4.5 to 5.5 mEq/L.	
___ 4. Causes of decreased calcium.	e. EDTA. f. Ferro and Ham.
___ 5. Level will be increased when calcium level is decreased.	g. Sulkowitch test. h. Heparin.
___ 6. Deficient in rickets.	
___ 7. Interferes with chemical determination of calcium by titration or colorimetric methods.	
___ 8. Method of analysis in which calcium is precipitated as insoluble salt of chloranilic acid.	
___ 9. Calcium in urine is precipitated with oxalic acid reagent and the degree of turbidity is noted.	
___ 10. Do not precipitate with calcium in the test for urine calcium.	

208. Identify the method, principle, reagent, and conditions for phosphorus determination.

Inorganic Phosphate. Like calcium, most phosphorus in the body is present in the skeletal system and teeth. Phosphorus also has a key role in the liberation of energy by what is termed the "high energy phosphate bond." The hydrolysis of adenosine triphosphate (ATP) produces about 8000 calories per molecule. In a discussion of biological energy, it is customary to represent such a high energy bond with a curved line (~), but the energy is not actually all contained in one bond. Rather, energy is released by group transfer as ATP is hydrolyzed to produce adenosine diphosphate (ADP). This is the mode of storing energy from the oxidation of fats and sugars, and in this sense phosphorus is the powerhouse of the body. In fact, phosphorylation is essential for intestinal absorption of various dietary components, particularly carbohydrates. The normal serum phosphorus level is 3.0 to 4.5 mg-%; however, with infants to 1 year of age, it may go up to 6.0 mg-%.

Normal levels. As previously indicated, there is a relationship between calcium and phosphorus in the body. As calcium levels rise, phosphorus levels normally decrease. In diabetic acidosis the inorganic phosphorus level of the blood increases, but phosphates may be administered by the physician to correct a deficiency of phosphate. Inorganic phosphorus level is sometimes low in rickets, as mentioned in the discussion of calcium, in which case the calcium is also decreased. Ordinarily, calcium and phosphorus bear an inverse relationship to each other. Acidosis of renal diabetes is different from acidosis of diabetes mellitus with respect to phosphorus. In renal disease, phosphate retention contributes to acidosis. To extend the anaology, in hyperparathyroidism there is a decrease in phosphorus as calcium increases. (This was also mentioned in our discussion of calcium.) It is difficult to discuss calcium and phosporus separately because levels of phosphorus necessarily relate to calcium levels. The discussions of clinical areas are by no means complete, but will reinforce your awareness of the need to consider each laboratory result in perspective.

Laboratory determination of phosphorus. Many phosphorus determinations are made using a trichloroacetic acid (TCA) filtrate. In the method of DRYER et al, Modified, which is outlined in AFM 160-49, the phosphate ion in a trichloroacetic acid filtrate, treated with molybdic acid reagent, reacts to form a phosphomolybdate complex. Quantitative reduction of the compound by p-semidine (N-phenyl-p-phenylenediamine) is accompanied by the formation of a blue color of an intensity proportional to the inorganic phosphate content. The molybdic acid reagent and the p-semidine reagent are easily decomposed. These reagents should be checked frequently for freshness. The method employs p-semidine as the reducing agent; p-semidine has high sensitivity, promotes rapid color development, and has a high degree of stability. Although most of the phosphorus in blood exists as *phosphates* or *phosphate esters,* because of the diversity of compounds, it is usually reported as phosphorus (P) but spoken of often as "phosphates."

Phosphorus measurements require a serum specimen free of hemolysis. The separation of serum from whole blood should be performed as quickly as possible after collection to preclude any contribution of serum inorganic phosphate from the large organic phosphorous fraction in erythrocytes. Heparinized plasma is not suitable for estimation of inorganic phosphate owing to the presence of this ion in commercial preparations of heparin.

Regardless of method, there are a few problem areas in performing phosphorus determinations. It is one of the few tests which are definitely affected by a nonfasting condition of the patient. Perhaps more important is the fact that whole blood samples are not stable. Intracellular phosphates hydrolyze and may, within a few hours, increase the serum phosphorus level by 100 percent. If the cells are separated from the serum, the specimen should be stable in the refrigerator for several days. Another potential source of error is the use of cleaning compounds which contain phosphorus. The possibility of contamination should always be considered if phosphorus results are elevated.

Exercises (208):

Match the following by placing the letter of the column B item beside the column A item or items that most nearly describe it. Each item in column B may be used once, more than once, or not at all.

Column A

___ 1. Energy released by group transfer as ATP and hydrolyzed to produce this energy compound.
___ 2. Is essential for intestinal components, particularly carbohydrates.
___ 3. In the sense of storing bodily energy is considered the powerhouse of the body.
___ 4. Normal serum values: 3.0 to 4.5 mg-%.
___ 5. Decreases as the calcium level rises.
___ 6. Condition in which the inorganic phosphorus level increases.
___ 7. Reducing agent in Dryer et al method for inorganic phosophorus.
___ 8. Condition in which most of the phosphorus exists in the blood.
___ 9. Measurement requires a specimen free of hemolysis.
___ 10. Not suitable for inorganic phosphate determination due to the presence of this ion in commercial preparations.
___ 11. One of the few tests definitely affected by a nonfasting condition of the patient.
___ 12. Should always be considered if phosphorus results are elevated.

Column B

a. ATP.
b. ADP.
c. Phosphorylation.
d. Magnesium.
e. Calcium.
f. Phosphorus.
g. Diabetic acidosis.
h. Phosphates or phosphate esters.
i. p-Semedine (N-phenyl-p-phenylenediamine).
j. Heparin.
k. Possibility of contamination.

209. Identify the method, conditions, sources of errors, and normal values for magnesium and lithium determinations.

Magnesium. The magnesium content of the red cells is about three times that of the plasma or serum; hence, hemolysis should be avoided. The intermediary metabolism of magnesium resembles that of phosphorus; both are present in bone and within tissue cells. Magnesium governs neuromuscular irritability and is important for coenzymes in the metabolism of carbohydrates and proteins.

Abnormal levels. Magnesium deficiency is known to occur in severe malabsorption states and after prolonged periods of intravenous therapy with magnesium-free solutions. Excess magnesium acts as a sedative and extreme excesses may cause cardiac arrest. Magnesium level will rise along with potassium in uremia. The normal levels of magnesium in the serum of adults is 1.5-2.2 mEq/L (1.8-2.6 mg/100 ml, since mEq/L × 1.2 = mg/100 ml).

Magnesium determination. Magnesium can be determined by atomic absorption spectroscopy, using methods similar to those for calcium analysis. It is difficult to determine magnesium by flame photometry because of the extremely hot flame required.

Lithium. Lithium ion in the carbonate form (Li_2CO_3) is used in the treatment of some psychiatric disorders. Since the range between the therapeutic and toxic levels is not large, it is necessary to adjust the dosage by monitoring the serum lithium level. Maintenance dose subsequently is 300 mg three times a day to give serum levels of 0.5 to 1.0 mEq/L of serum considered to be therapeutic levels.

Lithium analysis can be performed by either flame photometry or atomic absorption spectrophotometry. The atomic absorption is preferred and considered the reference method. When flame photometry is used, lithium standards should contain approximately normal serum concentrations for sodium, potassium, and calcium since lithium emission is reduced significantly without these other ions. Reduced emission standards tend to produce false elevations of patient lithium values. Accurate and precise estimation is available by the atomic absorption.

Exercises (209):

Match the following by placing the letter of the column B item beside the number of the column A item or items that most nearly describe it. Each element in column B may be used once, more than once, or not at all.

Column A

___ 1. Content in the red cells is about three times that of plasma or serum; hemolysis must be avoided.
___ 2. Used in the treatment of psychiatric disorders.
___ 3. Intermediary metabolism resembles that of phosphorus.
___ 4. Method preferred for lithium analyses.
___ 5. Value in mg-% if the magnesium level is 3.4 mEq/L.
___ 6. Governs neuromuscular irritability.
___ 7. Is important for coenzymes in the metabolism of carbohydrates and proteins
___ 8. Deficiency is known to occur in severe malabsorption states.
___ 9. Analysis can be performed by either flame photometry or atomic absorption spectroscopy.
___ 10. Reduced emission standards tend to produce false elevations of patient values.
___ 11. Lithium standards should contain approximately normal serum concentrations for sodium, potassium, and calcium when this method of analysis is used.

Column B

a. Lithium.
b. Magnesium.
c. 4.08 mg-%.
d. 6.08 mg-%.
e. Atomic absorption spectroscopy.
f. Flame photometry.

Liver Function Tests

THE FUNCTIONS of the liver are numerous and complex. Certain well defined tests in the clinical laboratory have become more or less routine as liver function tests. Many of these tests have been abandoned after early study. However, a few have been clinically useful. Among the most common are bilirubin and BSP. Thymol turbidity, once useful in detecting hepatic and nonhepatic diseases, is now less widely used than before. Some enzyme tests are classified as liver function tests, but will be discussed in a later chapter. Cholesterol is included in this chapter because it is formed in the liver and sometimes used as a diagnostic aid in liver disease. Cholesterol is not ordinarily considered part of a battery of liver function tests.

It is not suggested that tests discussed in this chapter are necessarily limited to liver functions or that the list of tests in this chapter is complete. Any laboratory test may, and frequently does, serve more than one purpose.

2-1. Physiological Basis of Liver Function Tests

If you have some idea of how the liver functions, you will gain an insight into the purpose and basis of liver function tests. The complexity of liver functions relates to equally complex biochemistry. As a clinical chemistry technician, you are expected to know the nature of the tests you perform for liver function. This implies an elementary understanding of the liver itself.

210. State the main functions of the liver and its associated structures.

Anatomy and Physiology of the Hepatic System. The liver is the largest organ of the body, with an average weight of 1500 gm in the adult male. It has two main lobes and is located in the right hypochondrium extending across the epigastrium to the left hypochondrium. Locate the liver and its associated organs in figure 2-1. The upper portion of the liver is overlaid by the lungs (pleura) and diaphragm. The lower portion overlaps the stomach and intestines. The liver is covered by a collagenous capsule which extends along the structures that enter it. The intralobular bile duct runs between the liver lobules which make up the lobes of the liver. Connective tissue of the liver supports the hepatic portal vein, which brings blood containing food absorbed in the intestine. Besides the bile ducts and the portal vein there are also the lymphatics and the portal artery.

Briefly stated, the major functions of the liver are: (1) formation of blood constituents such as prothrombin and destruction of red blood cells; (2) detoxification of harmful substances; (3) metabolism; (4) secretion of substances such as bilirubin conjugates, cholesterol, and dyes; (5) circulation of blood from the portal system; and (6) storage of glycogen and metabolic intermediates.

The liver may be described as both an endocrine and an exocrine gland. It is an endocrine gland because it secretes useful substances into the bloodstream. (The term "endocrine" is not restricted to the secretion of hormones which it receives from other glands of the body.) An exocrine secretion of the liver is, of course, bile. The liver is also antiendocrine in the sense that it removes hormones from the blood. An adult liver secretes 500 to 600 ml of bile in 24 hours. When liver tissue is destroyed, it demonstrates a remarkable capacity to regenerate. Interstitial inflammation with contraction of the liver tissue is referred to as *cirrhosis.*

The gallbladder is an organ extending from the bile duct which stores and concentrates secretions of the liver before they pass into the duodenum. The entire extrahepatic bile system is composed of hepatic ducts, the common bile duct, the cystic duct, and the gallbladder itself. The common hepatic duct is formed from a juncture of the right and left hepatic ducts and is also joined to the cystic duct. The gallbladder is attached to the lower side of the liver and is about 10 cm long and 5 cm in diameter. Histologically, the gallbladder consists of a folded mucous layer of cells, a muscular layer, and two delineated serosal layers of cells.

The physiological significance of bile is directly related to digestion and absorption of food. It activates lipolytic and proteolytic enzymes from the intestine and pancreas and emulsifies fats. Bile also functions in an excretory capacity, particularly with regard to cholesterol which is excreted in the bile as cholic acid. The discharge of bile into the intestine is controlled by two mechanisms. The first is the nervous system, and the second is activation by the hormone, cholecystokinin. Removal of the gallbladder surgically is accomplished by a procedure termed

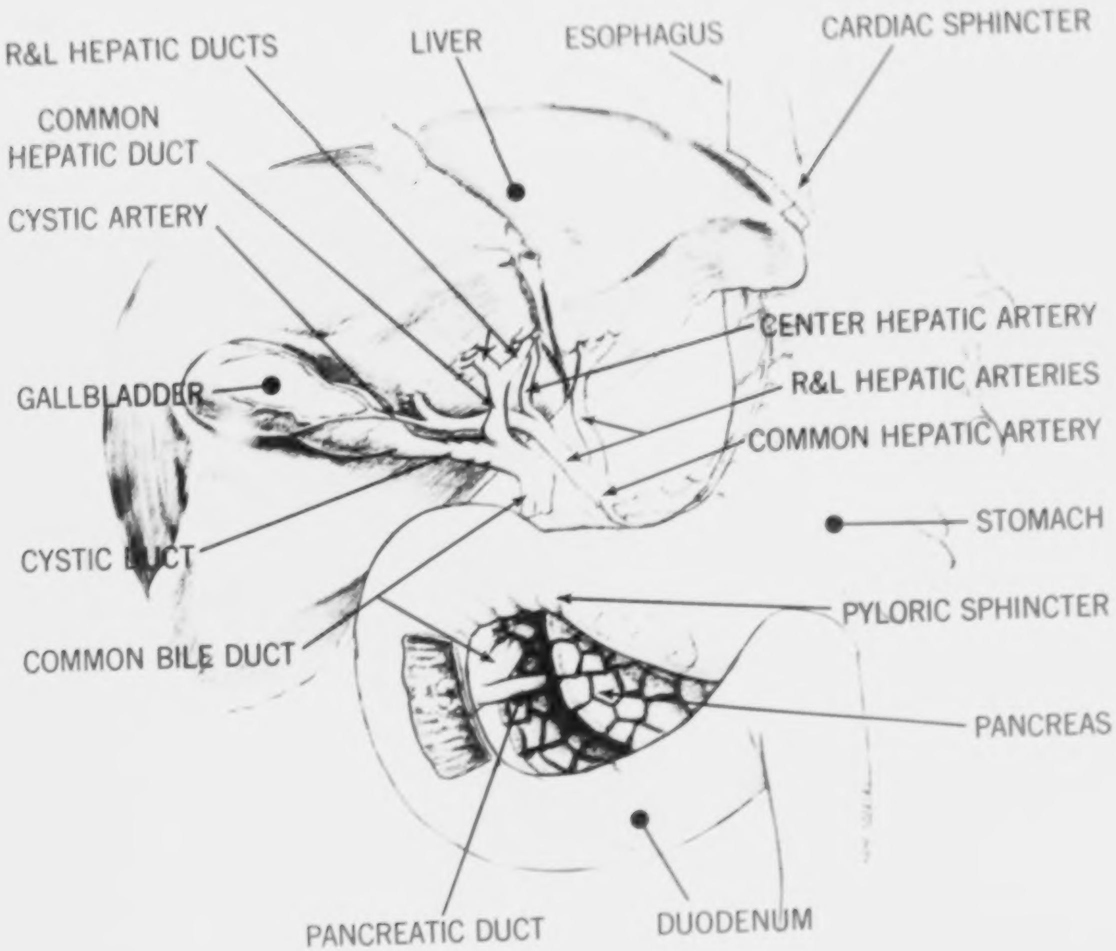
R&L HEPATIC DUCTS
LIVER
ESOPHAGUS
CARDIAC SPHINCTER
COMMON HEPATIC DUCT
CYSTIC ARTERY
CENTER HEPATIC ARTERY
GALLBLADDER
R&L HEPATIC ARTERIES
COMMON HEPATIC ARTERY
STOMACH
CYSTIC DUCT
PYLORIC SPHINCTER
COMMON BILE DUCT
PANCREAS
PANCREATIC DUCT
DUODENUM

Figure 2-1. The liver and associated organs.

"cholecystectomy." Following surgery, the bile ducts assume some of the activity of the gallbladder.

Bile Pigments and Related Pathology. In the normal formation of bile pigment by the liver, the destruction of red blood cells results in the production of biliverdinglobin, which is taken up by the reticuloendothelial system as well as by the Kupffer cells of the liver. In these particular cells the formation of indirect bilirubin takes place. Upon further modification by cells of the liver, indirect bilirubin is transformed into direct-reacting bilirubin. Once the direct bilirubin passes into the biliary passages it may be oxidized to biliverdin, which is green. Finally, direct-reacting bilirubin may reach the intestines, where intestinal bacteria reduce it to urobilinogen. Urobilinogen is made up of both mesobilinogen and stercobilinogen. Bilirubin, as such, does not appear in the feces except in pathological conditions. Most of the pigment of feces is derived from the oxidation of urobilinogen into urobilin. It is noted that resorption takes place in the intestine. This includes the resorption of urobilinogen as well as other constituents from the bile. Normally, only a small amount of bilirubin is present in the blood and will not appear in the urine except in pathological conditions. A small amount of urobilinogen is released in the liver and appears in the urine.

A disturbance in the formation or flow of bile will result in *jaundice*, a syndrome characterized by pigmentation of the skin. Consider first the jaundice which is not due to an obstruction of bile flow. Overproduction of bile as manifested in hemolytic jaundice is one type of nonobstructive jaundice. Due to overproduction, there is an excess of biliverdinglobin which leads to an excess of indirect bilirubin. The direct bilirubin remains normal, and no bilirubin will appear in the urine. Urobilinogen, however, is increased, and if increased sufficiently will result in an elevated urine urobilinogen. A second type of nonobstructive jaundice is called "retentive jaundice," manifested by an increase in the indirect bilirubin of the blood without an increase in urobilinogen.

Now let us consider the types of jaundice in which there is an impairment of bile flow. The obstruction may be complete or incomplete, intrahepatic or extrahepatic. In complete extrahepatic obstruction, there is a marked rise in the indirect bilirubin, with some rise in the direct-reacting bilirubin. The urine urobilinogen is negative because no bile reaches the intestine. If the obstruction is incomplete, as is frequently the case with gallstones, some bilirubin will pass into the intestine. Less urobilinogen is produced, but an increase of urine urobilinogen may occur due to maltransformation of urobilinogen by the liver. Intrahepatic obstruction is due to changes within the liver. In liver cell degeneration, serum bilirubin is high and bilirubin appears in the urine. Intestinal urobilinogen is decreased, but urine urobilinogen is increased due to maltransformation of urobilinogen in the liver.

The interpretation of laboratory findings in various types of jaundice is, of course, a clinical matter and is not within the province of the laboratory. However, you can appreciate from the preceding discussion how liver function tests can aid a diagnostician in separating forms of liver disease, such as the types of jaundice. It should be clear to you that bile pigments are all derived from the breakdown of hemoglobin and involve fairly complex chemistry. The free (indirect) bilirubin which is formed in the reticuloendothelial system is conjugated with glucuronic acid in the liver to produce direct bilirubin. The bilirubin is preceded by the formation of biliverdin. Of all the bile pigments, the clinical laboratory is primarily concerned with serum bilirubin, urobilin, and urobilinogen.

Exercises (210):

1. What is the chief exocrine secretion of the liver?

2. What are the primary functions of the gallbladder?

3. Describe the physiological significance of bile.

4. Why is it abnormal to find bilirubin in the feces?

5. Why is bile green?

6. How do you explain the fact that urine urobilinogen may increase in cases of obstruction where bilirubin available to the intestine for conversion of urobilinogen is diminished?

7. What is the source of bile pigments?

8. How is free (indirect) bilirubin formed?

211. Indicate how cholesterol is formed, excreted, and changed into esters in the body; state how much of the body cholesterol is in the form of esters and how liver damage affects the level.

Cholesterol. A significant function of the liver is the production of cholesterol. It is apparently synthesized from acetate ions and later excreted principally as cholic acid. Because of the importance of cholesterol in clinical medicine, we will consider it in some detail. Cholesterol is a white crystalline substance first isolated from gallstones, which sometimes form in the gallbladder and bile passages. It is a monohydroxy alcohol with the formula $C_{27}H_{34}OH$, and one double bond, as shown in figure 2-2. Slightly more dense than water, cholesterol has a molecular weight of about 386 and is relatively insoluble in water. Cholesterol may also be classified as a lipid, which is a category of organic compounds soluble in fat solvents. It is a sterol, characterized by the cyclic structure of the phenanthrene ring. Cholesterol accounts for about 90 percent of the steroids of the body. Hormones are also steroids and closely related to cholesterol, although cholesterol is not a hormone.

One fundamental category of reactions which cholesterol enters into is the formation of *esters*. Esterification of cholesterol is a chemical reaction in which the OH group of cholesterol is replaced by the alkoxy group of an organic acid. About 75 percent of the cholesterol in the body is in the form of esters. Esterification in the body is catalyzed by an enzyme of the pancreatic juice called cholesterol-esterase. That which is not in the form of an ester is termed "free cholesterol." It has recently been suggested that cholesterol may exist in the serum in fractions other than free cholesterol, or esters.

The diagnostic significance of ester levels has become a matter of questionable importance in many medical facilities. Separating the free cholesterol from the ester usually involves precipitating the free cholesterol as an insoluble compound with a plant extract which is a saponin called *digitonin*. Knowledge of the free cholesterol to esterified cholesterol ratio does have diagnostic significance. The question is whether this same information can be obtained from

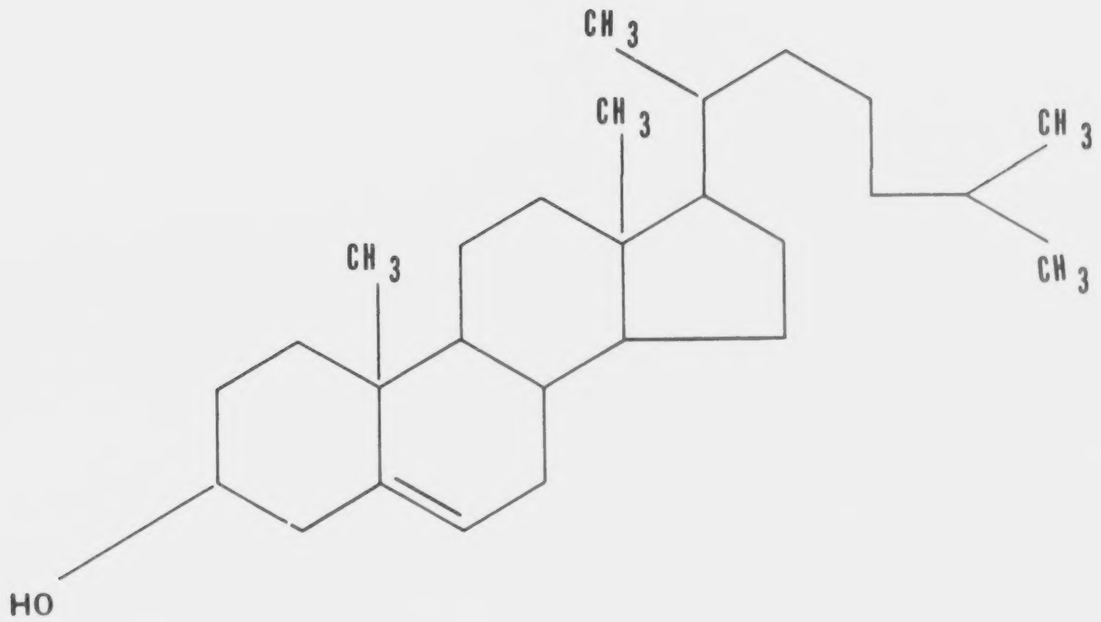

Figure 2-2. Cholesterol ($C^{27}H^{45}OH$).

other tests, whether the application is sufficient to warrant routine performance of cholesterol esters analysis.

Functions of Cholesterol. As stated earlier, total cholesterol is most significant to the clinician because of its direct relationship to liver cell damage and its more or less understood relationship to vascular diseases, such as atherosclerosis. The following functions are among those attributed to cholesterol:

a. It forms a large portion of tissue structure, particularly the brain.

b. It is considered a precursor of hormones, since all adrenal hormones are derived from cholesterol.

c. Evidence suggests it can detoxify certain agents in the blood.

d. It enhances antigen activity, probably by absorption, to increase surface area.

e. Cholesterol functions in fat transport.

f. It is a constituent of bile salts that emulsify fats.

Cholesterol is not a source of energy as are fats and carbohydrates. Since cholesterol is formed in the liver, it tends to be lower in cases of liver cell damage. Cholesterol originates from the diet, by synthesis in the liver, and to a lesser extent, in other body cells.

Exercises (211):

1. How is cholesterol formed and how is it excreted?

2. How are cholesterol esters formed in the body?

3. What percent of the body cholesterol is in the form of esters?

4. What is the level of cholesterol in cases of liver damage?

In exercises 5-18, indicate whether the following statements are true (T) or false (F). If you indicate that a statement is false, explain your answer.

T F 5. Cholesterol has a molecular weight of about 386 and is relatively soluble in water.

T F 6. Cholesterol may also be classified as a lipid, which is a category of organic compounds soluble in fat solvents.

T F 7. Cholesterol accounts for 10 percent of the steroids of the body.

T F 8. About 75 percent of the cholesterol in the body is in the form of esters.

T F 9. Cholesterol which is not in the form of an ester is termed "free cholesterol."

T F 10. Knowledge of the free cholesterol to esterified cholesterol ratio is of great diagnostic significance.

T F 11. Cholesterol forms a large portion of tissue structure particularly in the brain.

T F 12. Cholesterol is least considered in the formation of hormones.

T F 13. Cholesterol can detoxify certain agents in the blood.

T F 14. Cholesterol enhances antigen activity to increase surface area.

T F 15. Cholesterol functions in carbohydrate transport.

T F 16. Cholesterol is a constituent of bile salts that emulsify fats.

T F 17. Cholesterol is a source of energy as fats and carbohydrates.

T F 18. Cholesterol originates from diet, synthesis in the liver and body cells.

2-2. Laboratory Tests of Liver Function

Your competency as a technician is not only shown by your ability to perform the tests, but also by your ability to discuss the relationship of these tests to each other. You must carefully research the relevancy of the test to be used as a standard procedure in your laboratory. With the many procedures available, liver function tests must be given much concern, depending on their uses in clinical and preventive medicine.

212. Point out the principle, procedures, advantages, and disadvantages of the Malloy-Evelyn and Jendrassik and Grof methods for bilirubin determinations.

Bilirubin. As discussed earlier in this chapter, bilirubin is a product of hemoglobin breakdown and is excreted by the liver. Much confusion as to the actual processes involved in bile pigment metabolism still exists, but different forms of bilirin apparently are present in the plasma with different types of jaundice.

It is now generally accepted that bilirubin exists in the serum in two forms—as a free form, presumably absorbed on protein, and as a conjugated form, chiefly in glucuronates. The so-called direct-reacting bilirubin, the conjugated form, is more soluble in water and reacts relatively rapidly in aqueous solution. The free bilirubin is much less soluble in water and does not react, or reacts very slowly, in simple aqueous solution.

Malloy-Evelyn test. Bilirubin combines with diazonium salts to form azo dyes. This is the chemical basis of the Malloy-Evelyn test for serum bilirubin. For many years it has been known that not all of the bilirubin present in serum reacts at once with the dizao reagent in an aqueous solution. The addition of a fairly high concentration of methanol or ethanol as a solubilizing agent is necessary. A concentration of 40 to 50 percent methyl alcohol will dissolve the bilirubin sufficiently to react with diazo reagent. You may note that this is the basis of the commonly used Malloy-Evelyn method outlined in AFM 160-49, *Clinical Laboratory Procedures—Chemistry and Urinalysis.*

Jendrassik and Grof method. The alternate method of Jendrassik and Grof, also outlined in AFM 160-49, uses a solution containing a relatively high concentration of caffeine and sodium benzoate to dissolve the free bilirubin for a reaction. The principle of Jendrassik and Grof bilirubin method indicates that serum or plasma is added to a solution of sodium acetate and caffeine-sodium benzoate. The sodium acetate buffers the pH of the diazo reaction while the caffeine-sodium benzoate accelerates the coupling of bilirubin with diazotized sulfanilic acid.

Comparison of bilirubin determination methods. Despite its wide use, the Malloy-Evelyn test has several disadvantages that are not always kept in mind. The concentration of alcohol in the reaction for total bilirubin often causes some turbidity that is not always the same in the diazoptized sample as in the serum blank. The presence of more than traces of hemoglobin will interfere with the color reaction. In addition, the diazotized bilirubin is actually an indicator in which the color changes with the pH. The solution is only slightly buffered so that changes in pH can occur, particularly if the serum used is one in which the pH has changed on standing. The method of Jendrassik and Grof suffers less from this disadvantage. This procedure has the following advantages:

a. Insensitive to pH change in the sample.

b. High diazo color.

c. Stable color.

d. Insensitive to hemoglobin concentration up to 750 mg per dl.

e. Insensitive to a fifty-fold variation in protein concentration.

It is an excellent procedure for pediatric bilirubins because as little as 5 ul of serum can be used.

Bilirubin stock standard. It is recommended that a commercial standard such as Dade Bilirubin Control be used as a standard in the standardization procedure. This standard must contain a weighed amount of bilirubin in serum. A pooled serum commercial standard containing *assayed* value of bilirubin is *not* acceptable as a standard. Factors of time, temperature, and light must be controlled since bilirubin color fades significantly in the light at room temperature.

Exercises (212):

1. Based on the Malloy-Evelyn method for bilirubin determination, what reagent will dissolve bilirubin sufficiently to react with diazo reagent? What concentration of this reagent is required?

2. What are three disadvantages of the Malloy-Evelyn method for determination of bilirubin?

3. Briefly state the principle of the Jendrassik and Grof test for bilirubin.

4. What is the purpose of the sodium acetate in the Jendrassik and Grof procedure?

5. What are four advantages of the Jendrassik and Grof bilirubin method?

213. Indicate the procedures used in performing manual and automated determinations of total cholesterol by citing component reagents, reasons for variations of results, and sources of error.

Cholesterol Procedures. We will now consider the common test procedures for determination of chlesterol. There are several different reagents that are used for the determination of cholesterol. A common one is some modification of reagents used for the Lieberman-Burchard reaction, in which a mixture of acetic anhydride, acetic acid, and sulfuric acid gives a bluish green color with cholesterol. This method requires only the addition of reagents to serum and the measurement of the reasonably stable resultant color.

In essence, the most accurate methods for determining cholesterol involve extraction techniques, but even these involve a number of variables that must be considered. Fortunately, the normal values for a serum cholesterol represent a comfortable range within which some variation is expected by the clinician. The most recent figures list 144 to 350 mg/100 ml as normal for total cholesterol, with significant variations with respect to age and sex. The Schoenheimer-Sperry method, which uses the Lieberman-Burchard reaction, is not considered the reference method for cholesterol values.

The automation of a colorimetric procedure for cholesterol is based on a method introduced by Zlatkis, Zak, and Boyle in 1953. Much more sensitive than the Lieberman-Burchard reaction, this method results in a yellow color as cholesterol reacts with a mixture of concentrated sulfuric acid and ferric chloride ($FeCl_3$). A 1:10 isopropanol extract of serum is prepared and an aliquot of the extract is diluted with a segmented stream of premixed color reagent. Color is developed in a 95° C heating bath and measured at 520 mμ in a flow cell. Pulsing is effectively eliminated in the tubing by pulse chambers which are part of the Autoanalyzer system.

Other methods for the assay of cholesterol which do not use the Lieberman-Burchard reaction are not yet widely accepted but may someday become reference methods.

Cholesterol esters. Since the specificity is not absolute and the medical importance of cholesterol esters has declined as a sensitive test of hepatic function, this step is of historical interest only and may be reviewed for additional detail in most texts of laboratory methods.

Total cholesterol. A method for total cholesterol outlined in AFM 160-49, *Clinical Laboratory Procedures—Chemistry and Urinalysis,* agrees closely with the results obtained by that of Schoenheimer-Sperry. The principle indicates that total cholesterol in serum reacts with sulfuric acid in acetic anhydride in the presence of sulfosalicylic acid to form a stable green color. The color is proportional to the concentration of cholesterol. Reagents used in this procedure are:

a. Acetate acid, Glacial, AR.

b. Sulfosalicylic acid, 12 percent, 30 gms of analytic grade sulfosalicylic acid dissolved in 250 ml of glacial acetic acid.

c. Sulfuric acid Concentrated, AR.

d. Cholesterol, standard, 200 mg per dl.

Variation in results. A variety of methods for preparing compensating blanks can be found in current literature. Experiments demonstrate that 10 mg-% of bilirubin can elevate a cholesterol result by as much as 200 mg-%. This means an increase of 100 percent over the normal values generally accepted for cholesterol. This is a significant error, which has until recently been discounted in many technical publications. The interference of bilirubin and complexities of the color reaction are responsible for variation in cholesterol results, even among laboratories staffed by qualified analysts.

Sources of error. Sources of errors are:

a. High bilirubin or hemolized specimen.

b. Failure to mix when this step is indicated or mixing when this step is contraindicated.

c. Failure to strictly adhere to the order of addition of reagents.

Exercises (213):

1. What are the essential components in the Lieberman-Burchard reaction?

2. What are the essential components in the Schoenheimer-Sperry method for cholesterol?

3. What are two given causes for the variation of cholesterol results?

4. What are three sources of errors?

214. Given a list of the various liver function screening tests, identify them as to reagents, procedures, and possible sources of errors.

Liver Function Screening Tests. A few relatively simple tests are frequently used to aid the physician in evaluating the general condition of the liver. Some widely used tests for this purpose are thymol turbidity, cephalin-cholesterol flocculation, and the bromsulphalein retention test (BSP).

Thymol turbidity test. Turbidity is caused in a buffered thymol solution when a serum that contains an abnormal globulin is added to the solution. The degree of turbidity is measured photometrically at 650 mμ, and the degree of turbidity reported in Shank-Hoagland units. The standard consists of a barium sulfate suspension which is prepared from 0.0962M barium chloride and dilute sulfuric acid. The buffer is a thymol-barbital buffer which should have a pH of 7.55. If a pH of 7.8 is used, the results will be 30 percent lower. A Shank-Hoagland unit is equivalent to one Maclagan unit. However, it should be pointed out that units are reported by some medical facilities in which one Maclagan unit equals two Shank-Hoagland units because they may use a standard that is 0.0962N instead of 0.0962M. Thus, clinicians may be familiar with a Shank-Hoagland unit as representing two rather than one Maclagan unit. It should be noted that values listed for many commercial controls are in Shank-Hoagland units.

Cephalin-cholesterol flocculation. Normal serum will not flocculate an emulsion of cephalin, cholesterol, and lipid which is prepared according to the method of Hanger. In liver damage there is an alteration in the protein fraction of serum which causes precipitation that can be quantitatively estimated when serum and antigen are mixed. There is no high degree of standardization among lots of antigen, and no other method of standardization is available. However, the test is reproducible for any lot of antigen. The exact nature of protein changes which are responsible for flocculation are not yet agreed upon. It is most likely due to an increase in the gamma globulin fraction and a decrease in the albumin fraction.

High serum lipids do not interfere with the cephalin-flocculation test as they do with the thymol turbidity test. The cephalin-cholesterol emulsion is commonly referred to a cephalin-flocculation antigen. The term "antigen" is not completely accurate in describing the cephalin-flocculation reagent because the reaction does not involve antibodies. We usually define an antigen as a substance which causes the formation of antibodies, and this has no application to the emulsion described. The test is usually read as 1+, 2+, 3+, or 4+ in 24 and 48 hours.

According to some investigators, an abnormal finding would appear within 24 hours and there would, therefore, seem to be little justification for submitting a 48-hour reading. In fact, a reading at 37° C at the end of 3 hours has been reported to correlate within acceptable limits with the 24-hour reading.

Stability of the cephalin-cholesterol emulsion depends to a great extent on the way in which it is prepared. Since the working emulsion is not usually sterile, it should be prepared fresh, as it deteriorates. However, the emulsion as originally prepared is sterile; it is stable for several months if sterility is maintained during refrigerator storage. If no attempt is made to maintain sterility, the emulsion, if stored in the refrigerator, is usable for 1 to 4 weeks. Other sources are more optimistic, but all agree it should not be kept for more than a few weeks and that it should be refrigerated and checked for bacterial and mycotic contamination. All of the well-known sources of error for this test, such as dirty glassware and emulsion preparation, must be recognized for this to be a valid test.

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Foreign substance excretion. For many years it has been recognized that abstraction of foreign dyes from the blood by the liver can be applied to the testing of hepatic function. There are three dyes used for this purpose: rose bengal, indocyanine green, and sulfobromophthalein (BSP). The BSP excretion test is a standard and widely used test of hepatic function. The former tests are somewhat rarely used in the application of clinical medicine.

BSP. The BSP test is one which measures the *retention* of bromsulphalein dye by the liver following intravenous injection. The patient is usually in the fasting state when BSP dye is injected. Injection is accomplished by a physician and is not usually undertaken by support personnel or laboratory technicians, because very serious reactions to BSP dye, including death, have been reported.

It is customary for laboratory personnel to assist the physician by preparing the syringe and dye. The syringe used to inject the dye must be sterile. The needle used to fill the syringe should be discarded and replaced with a new one prior to injecting 5 mg of dye per kilogram of body weight. The conversion to milliliters of dye can be readily obtained from a chart supplied by pharmaceutical firms who provide the dye, or a simple calculation can be used. The patient's weight in pounds divided by 22 is equivalent to the milliliters of 5 percent dye to inject.

Blood is withdrawn from the patient at a particular interval thereafter (usually 45 minutes) and serum is used for the test. Whether the vein into which the dye was injected is used as the site for withdrawing blood, or whether the opposite arm is used is of relatively minor importance. The procedure then allows for color development with an alkaline solution and calculation of percent retention with a standard or from a prepared curve.

The test is based on the assumption that most BSP dye in the body is removed by the liver and thus serves as an index of liver excretory function. Any blockage of bile flow will impair the ability of the liver to excrete BSP dye. Hence, an increased serum bilirubin, if due to obstruction, will be accompanied by an abnormal BSP test. Photometric interference by icteric serum does not occur significantly if the bilirubin concentration is less than 20 mg-%.

Exercises (214):

Match the following by placing the letter of the column B item beside the number of the column A item or items that most nearly describe it. Each element in column B may be used more than once or not at all.

Column A

___ 1. Standard consists of barium sulfate suspension prepared from 0.0962M barium chloride and dilute sulfuric acid.
___ 2. Results will be 30 percent lower if pH is 7.8.
___ 3. Not affected by high serum lipids.
___ 4. Stock antigen is considered stable for several months if sterility is maintained during refrigerator storage.
___ 5. Dye injection is usually accomplished by physician.
___ 6. Reagent subject to bacterial and mycotic contamination.
___ 7. Procedure allows color development with an alkaline solution.
___ 8. Called retention test because report is in percent retained rather than percent excreted.

Column B

a. Thymol turbidity test.
b. Cephalin-cholesterol flocculation.
c. BSP.
d. Rose bengal excretion.
e. Indocyanine green.

Proteins

PROTEIN CHEMISTRY is a vast and complex study. The relevance of proteins to body functions is so extensive that there appears to be no limit which could be clearly set for the clinical chemist in the area of protein chemistry. All body cells, as well as the intercellular material, contain proteins. Hemoglobin, enzymes, and most hormones are protein in nature, as are the immunological mechanisms of the body. This chapter is limited to a few protein determinations that are routinely performed in the clinical laboratory. The changes which proteins undergo in various clinical conditions are far more extensive than can be resolved by a few relatively simple laboratory tests. This chapter is intended to give you background information which will be used in later chapters of this course and in other CDCs for which this course is a prerequisite. More detailed protein measurements will be discussed in a later chapter in connection with the technique used. Enzymes are also discussed separately.

3-1. Chemistry and Physiology of Proteins

Much has been written in recent years about the chemistry and physiology of proteins. Proteins constitute a large portion of blood, muscle, and other tissue. All enzymes and most of the hormones are proteins. In this section we will briefly describe the chemical nature of proteins, followed by a discussion of the application of proteins to physiology and the practice of medicine.

215. Identify the essential characteristics relating to the chemistry, types, terms, and properties of proteins.

Chemistry of Proteins. Proteins are large molecules composed of chains of *alpha*, or essential *amino acids*. An essential amino acid is an alpha amino acid derived from proteins that is indispensable for the optimum growth of the animal body and must therefore be supplied in the diet. The general formula for an alpha amino acid is $R\text{-}CH(NH_2)\text{-}COOH$. In solutions at the proper pH, amino acids exist in an ionized form. That is, the carboxyl group (COOH) loses a proton to water so that a COO^- group is formed, and the amino group (NH_2) gains a proton. The amino group of an amino acid can react with the acid group (COO^-) of another amino acid to form a peptide linkage. Amino acids combine with each other in long chains, and each may be of the same structure, or different. Chains of amino acids are called polypeptides, the protein molecule being an elaborate polypeptide, as illustrated in figure 3-1.

The sequence and characteristics of the amino acids determine the nature of the proteins which they form. Keeping in mind that a protein molecule is three-dimensional, you should realize there is an almost infinite number of ways in which the molecule may be geometrically arranged. The exact arrangement is a significant factor affecting the nature of proteins. Scientists are able to study the dimensional structure of a protein molecule by various techniques using X-ray beams. As an X-ray beam is sent through a crystal from various directions, the pattern produced on a photographic plate will indicate the configuration of the molecules. From this information investigators are able to construct three-dimensional models of a protein molecule. The chains of molecules may be twisted about each other to form a helix pattern, which is characteristic of certain proteins, such as the collagen molecule diagrammed in figure 3-2. There are, of course, many other three-dimensional configurations. At the present time more than 20 different amino acids have been isolated from proteins upon hydrolysis. The possible ways in which over 20 amino acids can combine suggest a wide variety of proteins, and indeed there are. A change in the sequence or position of one amino acid alters the protein.

There are various ways of classifying proteins, though no system has been devised which is beyond criticism. Proteins that contain amino acids, or their

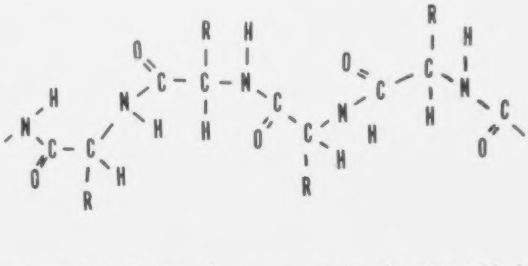


Figure 3-1. Portion of repeating chain of amino acids in polypeptide linkage.

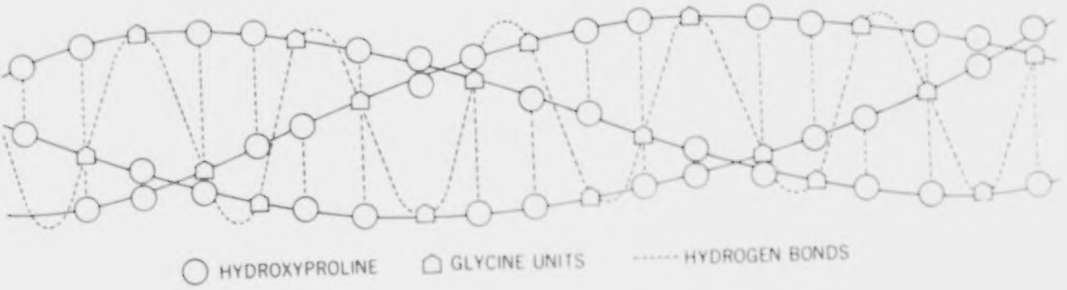


Figure 3-2. Diagram showing helix structure of a protein molecule.

derivatives, and no other substances are frequently classified as simple proteins. Those which contain a nonprotein substance, called a prosthetic group, are referred to as conjugated proteins. Albumin and globulin are simple proteins, whereas chromoproteins (hemoglobin), mucoprotein (mucin), phosphoproteins, and lipoproteins are examples of conjugated proteins. Because of the large size of a protein molecule, proteins behave as *colloids* in water. Colloids are particles held in aqueous suspension due to a marked attraction between the water molecules ad protein molecules. Suspended molecules of protein are also called hydrophilic or lyophilic colloids. Proteins have the interesting characteristic of changing charge—being electrically positive, negative, or neutral as conditions vary. Substances which act as both acids and bases because of a duality of electrical charge are described as *amphoteric* or *amphoterous* (from the Greek word meaning "both"). The protein molecule is amphoterous because it bears a charge dependent upon the pH of the solution in which the protein occurs. The electrical behavior of proteins will be further described in another chapter in the context of protein electrophoresis.

Properties of proteins were described in some detail as early as 1807 by the Russian investigator, Reuss. Then, in 1861 the migration of proteins in an electrical field was related to the pH of the solution. It was discovered later that electrical properties of proteins are conferred upon them by the alkaline or acid nature of the medium. The pH at which proteins are electrically neutral is called the *isoelectric point.* Obviously, the isoelectric point will be different for different proteins because all protein fractions do not have the same charge characteristics. You should recall that proteins remain in solution as a result of the attraction of protein molecules for each other and the attraction between the water molecules and protein molecules, as mentioned above. The greater the charge on the molecules, the less significant will be the force opposing suspension. At the isoelectric point, the net charge of the proteins will be zero and the protein molecules will, therefore, cease repelling each other. If the protein is in a salt solution of optimal concentration at its isoelectric point, the salt molecules will compete for the water and become hydrated. Conversely, the protein molecules will become dehydrated and precipitate. This is the chemical basis of "salting out" techniques which will be discussed later in this chapter. It is a useful principle that can be applied to separate, differentially, one protein from another in colloidal suspension.

Exercises (215):

Match each of the following characteristic chemistry, types, terms, and properties of proteins in column B with the conditions in column A which most nearly describe it. Each column B item may be used once or more than once.

Column A

___ 1. Albumin and globulin.
___ 2. Proteins which contain nonprotein, a substance; called a prosthetic group.
___ 3. Chromaproteins, mucoproteins, phosphaproteins and lipoproteins.
___ 4. Particles held in aqueous suspension due to a marked attraction between the water molecules and protein molecules.
___ 5. Basic composition of large molecule of proteins.
___ 6. Chains of amino acids.
___ 7. $R\text{-}CH(NH_2)\text{-}COOH$.
___ 8. Proteins which act as both acids and leases because of duality of electrical charge.
___ 9. If the protein is in a salt solution of optimal concentration at its isoelectric point, the salt molecules will compete for water and become dehydrated.
___ 10. The pH at which proteins are electrically neutral.

Column B

a. Alpha, amino acid.
b. Polypeptides.
c. Protein molecule.
d. Simple proteins.
e. Conjugated proteins.
f. Colloids.
g. Amphoterous.
h. Isoelectric point.
i. Chemical basis of salting out.

216. Identify the processes, terms, and conditions related to the normal and abnormal physiology of proteins.

Physiology of Proteins. Proteins that are taken into the body in the form of various foods begin to break down by the action of pepsin in the stomach. Breakdown of the large protein molecules in the stomach results in proteoses and peptones, as well as some amino acids, which are the basic units. Further breakdown of proteins, proteoses, and peptones occurs in the intestine under the action of the enzymes, trypsin, and chymotrypsin from the pancreas. Polypeptides which are not hydrolyzed to amino acids by the activity of other enzymes are broken down to amino acids by peptidase, including carboxypeptidase, also from the pancreas.

Proteins are absorbed into the blood in the form of amino acids and a few relatively short peptide chains. The absorption of large protein molecules may result in the production of antibodies as a systemic response. Amino acids absorbed into the blood are immediately taken up by the tissues with no appreciable increase in the plasma amino acid level. The amino acids are then used for protein synthesis, stored in a phosphorylated form, or deaminated in the liver. Some may be used for special purposes.

Proteins of the body are constantly being broken down and re-formed. The processes by which proteins are synthesized in the body are still undergoing investigation. It is sufficient to state here that protein metabolism is a dynamic process which is constantly taking place, and involves the transfer of energy by means of the high energy phosphate bond mentioned in an earlier chapter. Proteins may be used for energy if the intake of carbohydrates and fat is inadequate, or if a metabolic dysfunction occurs. In starvation there is a wasting away of muscle tissue as proteins of the muscle are catabolized to produce energy. In addition to the necessary amino acids for protein synthesis, dietary proteins furnish the amino acids necessary for the synthesis of various body compounds other than body proteins. For example, the amino acid, glycine, converts benzoic acid to hippuric acid, the latter being excreted by the kidney. As will be noted in a discussion of enzymes, amino groups are frequently transferred from one amino acid to another by the process of *transamination*. There are many possible ways of converting various amino acids as they are required by the body. Amino groups which are not transferred to an amino acid are converted into urea. Urea is excreted via the kidneys.

Amino acids, as such, are excreted only in trace amounts in the urine. Excretion of amino acids to any significant extent suggests a pathological condition, such as abnormal protein metabolism or tubular resorption problems. Many of the metabolic disorders are hereditary. One such common disorder familiar to laboratory technicians is phenylketonuria (PKU) which results from an accumulation of phenylalanine. If undetected very early in life, this metabolic disorder results in irreversible brain damage. The laboratory test for PKU is included in the volume covering urinalysis, but is worth mentioning here as a disorder of protein metabolism. Albinism, another disorder of protein metabolism, is, in some ways, analagous to diabetes, which is a disorder of carbyhydrate metabolism. A most interesting genetic trait associated with proteins is involved with sickle-cell anemia. In this inherited disease the substitution of one amino acid (valine) for another (glutamic acid), among over 500 amino acids in the molecule, produces the abnormal "S" hemoglobin.

Another relevant syndrome familiar to the laboratory worker is a tumorous condition of the intestinal tract characterized by the presence of 5-hydroxyindolacetic acid (5-HIAA) in the urine. The compound, 5-HIAA, is derived from serotonin which is an amino acid (5-hydroxytryptamine). Thus, we have another familiar example of faulty protein metabolism. Through the process of synthesis, the body is able to produce all but 8 of the 20 amino acids. These eight, sometimes called the essential amino acids, are: threonine, valine, leucine, isoleucine, methionine, lysine, tryptophane, and phenylalanine. If the diet is deficient in any of the eight amino acids listed, the individual will develop various symptoms of protein deficiency.

Biochemists have studied the structure of proteins much more extensively than the nature of molecules which participate in the body systhesis of proteins. One group of control molecules is the ribonucleic acid (RNA) compounds. RNA is a complicated chemical structure, formed from pentoses, a phosphoric acid, and nitrogen bases. RNA plays a crucial role in each step of the process of protein synthesis within the cells. The key to understanding how a nucleic acid molecule is able to control synthesis is the structure of the nucleic acid molecule itself. One of the first molecules studied in detail was one of the short RNA molecules which transports the amino acid, alanine. The nucleic acid components, known as nucleotides, were described in detail for this particular molecule and reported for the first time in 1965. When we consider that this is one of the protein synthesis control molecules structurally elaborated, we realize that the biochemistry of proteins requires many years of further study. The complex functions of another nucleic acid group called deoxyribonucleic acid (DNA) also continues to be sutdied extensively, particularly with respect to hereditary control. Other factors that control protein synthesis include the many enzymes which, as stated previously, are themselves proteins.

Exercises (216):

Match each of the following processes, terms, and conditions of normal and abnormal protein physiology in column B with the statement in column A which most nearly describe it. Each column B item may be used once or more than once.

Column A

___ 1. May be used for energy if the intake of carbohydrates and fat is inadequate.

___ 2. Not hydrolyzed to amino acids by the activity of other enzymes; are broken down to amino acids by peptidase.

___ 3. Causes further breakdown of proteases, proteins, and peptones in the intestines.

___ 4. The transfer of an amino group from one compound to another.

___ 5. Excreted via the kidneys.

___ 6. Not transferred to an amino acid; are converted to urea.

___ 7. A disorder of protein metabolism.

___ 8. Is in some ways, analagous to diabetes.

___ 9. An inherited disease in which there is a substitution of one amino acid (valine) for another (glutamic acid).

___ 10. Complicated chemical structure, formed from pentoses, a phosphoric acid, and nitrogen bases; and plays a role in each step of protein synthesis within the cells.

Column B

a. Trypsin and chymotrypsin.
b. Polypetides.
c. Proteins.
d. Transamination.
e. Amino group.
f. Urea.
g. Phenylketonuria.
h. Albinism.
i. RNA.
j. Sickle-cell anemia.

3-2. Laboratory Investigation of Proteins

Methods of protein analysis outlined in AFM 160-49, *Clinical Laboratory Procedures—Chemistry and Urinalysis*, are discussed in this section. Information concerning protein levels are of diagnostic value to the physician, although protein functions are so numerous and complex that other laboratory tests, as well as the usual clinical evaluations, may be necessary to establish a definitive diagnosis. Besides data concerning total protein and the A/G ratio, the physician may be concerned with other protein elements, such as fibrinogen or hemoglobin. A complete analysis of fractions, including albumin and globulin, is usually done by electrophoresis which will be discussed in a later chapter.

217. Identify the methods of determination, principles, reagents, procedures, and interpretation of results for total protein in serum, urine, and cerebrospinal fluid.

Biuret Method for Total Protein. Even though this method is not very sensitive and not suitable for analysis of specimens containing small amounts of protein such as urine and cerebrospinal fluid, the biuret reaction for the determination of serum protein is probably the most widely used procedure. The protein in serum is reacted with an alkaline copper sulfate to form a blue-violet complex (biuret reaction). Since interfering substances in serum are present in minimal amounts with large amounts of protein, the reaction is virtually specific for proteins. Several different modifications of the original biuret method are used today.

Formula. The formula recommended in AFM 160-49 for stock and working biuret reagent is as follows:

a. Biuret Stock Reagent. Dissolve 60.0 g of analyzed reagent grade potassium sodium tartrate (Rochelle salt) in about 500 ml of water in a 1-liter volumetric flask. Add 300 g of analyzed reagent grade sodium hydroxide pellets, and mix under running tap water until dissolved and cool. Add 15.0 g of cupric sulfate pentahydrate or the equivalent, and shake vigorously until completely dissolved. Dilute to the mark with water, and mix thoroughly by inversion. Store in a polyethylene bottle. Stable indefinitely.

b. Biuret Working Reagent. Dilute 50.0 ml of stock reagent to 500 ml with water in a volumetric flask. Mix well and store in a polyethylene bottle. Alternately dilute as required, and store in any of several forms of automatic dispensing systems.

A number of commercial preparations are available which have the name "biuret reagent." They all contain copper sulfate, but some may contain other stabilizing agents, such as citrate or NaOH instead of KOH. Increased stability has been reported by substituting Na_2CO_3 for the NaOH (or KOH) and then adding the hydroxide separately before using the working solution.

Problems. There are two distinct problems that must be overcome in stabilizing biuret reagent. The first is that $Cu(OH)_2$ tends to precipitate from solution. The second difficulty is that a highly alkaline solution of copper sulfate can be easily reduced if contaminated. In any event, if a stable biuret reagent is used, the biuret method generally produces satisfactory results. The biuret method may be applied to protein analysis in cerebrospinal fluid, but is less sensitive at lower protein concentrations. Urine proteins are not usually assayed by this method unless the protein is first separated from interfering substance by precipitation, or the modified biuret method is used. Bile pigments (bilirubin) are reported to interfere with this reaction only if their concentration is greater than 30 mg/%. Hemoglobin will react with biuret reagent if the specimen is hemolyzed.

The biuret reaction has been adapted to the Autoanalyzer. Many earlier biuret procedures contained potassium iodide to prevent autoreduction.

Results indicated that potassium iodide was unnecessary; thus, the biuret reagent without potassium iodide has also been used in automated procedures.

Precipitation Methods. The precipitation of proteins by acids to produce a turbid solution is a useful concept with urine and cerebrospinal fluid. This is not the technique used for serum, which requires a more precise method suitable for the relatively high concentration of protein in serum or plasma. In the classical method of Kingsbury and Clark for urine proteins, the protein was precipitated with sulfosalicylic acid after clearing the urine of insoluble phosphates with acetic acid. More recently, trichloroacetic acid (TCA) is substituted for sulfosalicylic acid. This is because sulfosalicylic acid tends to give strikingly different turbidities with albumin and globulin. Specifically, above 23° C, albumin gives much more turbidity than an equivalent concentration of globulin. Below this temperature, albumin yields less turbidity than globulin. Total protein results, therefore, become a function of the A/G ratio of the urine rather than the absolute amounts of albumin and globulin contained in the urine. This method was described in a procedure disseminated by the USAF Epidemiology Laboratory in 1965.

The turbidity produced is an indication of the amount of protein present in the specimen. Unfortunately, the protein particles flocculate rather rapidly after the precipitate is formed. To be measured reliably in the spectrophotometer, the precipitate should be fine, not a heavy flocculation. This problem may be overcome by breaking up the floc mechanically by shaking or by the addition of gum ghatti. If gum ghatti is used, a corresponding blank must also be used. A calibration curve is usually prepared using a suitable protein standard such as the standard available through regular supply channels. By measuring the 24-hour volume of urine, you can easily relate the concentration of an aliquot of the unknown to protein excretion per 24-hour period with the following formula:

$$\text{mg protein per 24 hr} = \text{mg-\% protein} \times \frac{\text{volume of 24-hr specimen}}{100}$$

You should make certain that a specimen submitted for a 24-hour analysis of any kind is in fact a 24-hour specimen. As a guide, you may keep in mind that the normal adult 24-hour urine volume is 1200 to 1600 ml. It is advisable to report the 24-hour volume together with the test results on the laboratory report form. Refrigeration or addition of toluene is usually adequate to preserve the specimen prior to analysis. The TCA precipitation method may also be applied to spinal fluid protein.

Many laboratories test cerebrospinal fluid (CSF) by the Pandy qualitative test for globulin on the assumption that any significant increase in globulin can be so detected. Recent studies have shown that solutions of phenol are unreliable and not specified for globulin. Hence, the Pandy globulin test has been abandoned by many progressive laboratories. The normal spinal fluid protein is from 15 to 45 mg-%. You might assume that since qualitative protein "sticks," used in screening urine specimens, are sensitive from about 30 mg-%, they would also be ideal for screening spinal fluid protein. Unfortunately, this is not the case, because spinal fluid proteins are approximately 50 percent globulins to which the strip indicator, tetrabromphenol blue, is much less sensitive. In fact, levels of globulin from 50 to 100 mg-% have not been reliably detected by this test. However, the usefulness of protein sticks in screening urine specimens is not precluded by this problem. Further, no manufacturer has ever advocated the use of this product for cerebrospinal fluid. A phenol method is currently used which involves a reaction between phenol and a phosphotungstic-phosphomolybdic acid reagent. It is a reasonably sophisticated procedure which is reported to be 100 times more sensitive than the biuret method. Sensitivity is, of course, a consideration with low protein values.

Exercises (217):

Match each of the following methods, principles, reagents, interpretation of results in column B with the column A statements which most nearly describe it. Each column B item may be used once or more than once.

Column A

___ 1. Should be mixed under running water until dissolved and cool.
___ 2. The protein in serum is reacted with an alkaline copper sulfate to form a blue-violet complex.
___ 3. Used as a stabililzing agent for biuret reagent.
___ 4. Will react with biuret reagent if the specimen is hemolyzed.
___ 5. Interfere with the biuret reaction if concentration is greater than 30 mg-%.
___ 6. Precipitation of protein in urine with sulfosalicylic acid after clearing the urine of insoluble phosphates with acetic acid.
___ 7. Method substituted for sulfosalicylic acid because sulfosalicylic acid tends to give strikingly different turbidities with albumin and globulin.

Column B

a. Biuret reaction.
b. Potassium sodium tartrate and sodium hydroxide.
c. Citrate and Na_2CO3.
d. Bilirubin.
e. Hemoglobin.
f. Kingsbury and Clark method.
g. Trichloroacetic acid (TCA).
h. Qualitative protein "sticks."

Column A

___ 8. Method may also be applied to spinal fluid protein.

___ 9. Method not suitable for qualitative screening of spinal fluid because, the indicator, tetrabromphenol blue, is much less sensitive to globulin.

___ 10. Levels of globulin from 50 to 100 mg-% have not been reliably detected by this method.

218. Indicate the methods of determination of total protein in urine and cerebrospinal fluid, reagents used, and source of errors.

Determination of Total Protein in Urine. The lowest normal values for urine proteins are obtained using turbidimetric and dye-binding procedures, while higher values are obtained using the biuret and other modifications of this method. The sensitivity and simplicity of the turbidimetric procedures have enabled them to gain widespread acceptance for determination of protein in urine. The biuret procedure for urine protein is outlined in AFM 160-49. The procedure requires precipitation of total proteins in urine with trichloroacetic acid. The washed precipitate is dissolved in a biuret reagent. The intensity of the red-violet color is measured in the spectrophotometer and the protein concentration is calculated. The procedures require that 10 percent trichloroacetic be added to a specific qualitative quantity of urine in pairs indicated on a chart. Allow the samples to stand for 5 minutes at room temperature, centrifuge, and discard the supernate. To one of each pair of unknowns add 20 ml of 0.75 M NaOH to dissolve precipitate and to remaining unknowns and a standard add to 2.0 ml of biuret working reagent. Then allow to stand at room temperature for 10 minutes. Reading in spectrophotometer is made at 555 nm. You must be aware that interfering substances exist in greater quantity in the urine than in cerebrospinal fluid. For example, the inorganic ion content is higher, and the protein levels are lower.

Determination of Total Protein in Cerebrospinal Fluid. In the procedure for total protein in cerebrospinal fluid outlined in AFM 160-49, total proteins are precipitated with trichloroacetic acid and dissolved in biuret reagent. The intensity of the red-violet color is measured spectrophotometrically and the protein concentration is calculated. In this procedure CSF protein is precipitated with 20 percent trichloroacetic acid by adding 1.0 ml of the TCA to 1.0 ml of clear CSF and working standard. The samples are allowed to stand for 5 to 10 minutes. Reading in a spectrophotometer is made at 555 nm. Failing to avoid foaming may alter readings and values given to samples for protein analysis.

Exercises (218):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

T F 1. The highest normal values for urine proteins are obtained using turbidimetric and dye-binding procedures.

T F 2. The washed precipitate is dissolved with TCA.

T F 3. Interferring substances exist in greater quantity in cerebrospinal fluid than in urine.

T F 4. The inorganic ion content is higher, and the protein levels are lower in urine.

T F 5. In the procedure for CSF protein, precipitation is accomplished by adding 1.0 ml of 20 percent TCA to 1.0 ml of clear CSF and working standard.

T F 6. Failing to avoid foaming may alter readings and values given to samples for protein analysis.

219. Indicate the procedures for determination of serum protein fraction in terms of reagents, methods, and the percentage of fibrinogen in the plasma.

Establishing the A/G Ratio. The best method of determining serum protein fractions is by electrophoresis. Relatively few Air Force laboratories are authorized the expensive equipment necessary for electrophoresis, and it is therefore desirable that a method be available to at least assay the albumin and globulin fractions. The clinical importance of the A/G ratio is well known, being of particular value in liver disease, congenital anomalies, and malignancies. Some diseases and their usual protein patterns are listed in table 3-1. The importance of determining more than just total protein should be clear to laboratory personnel, although clinical interpretation is not the

TABLE 3-1

CLINICAL CONDITIONS AND CHARACTERISTIC ALBUMIN-GLOBULIN FRACTIONS OF SERUM

CONDITION	TOTAL PROTEIN	ALBUMIN	GLOBULIN	A/G
Multiple myeloma	Increased	Normal	Increased	Decreased
Dehydration Hemoconcentration	Increased	Increased	Increased	Normal
Certain liver diseases	Normal	Decreased	Increased	Decreased
Kidney damage Malnutrition Malabsorption syndromes	Decreased	Decreased	Normal	Decreased

concern of the laboratory. You should know the normal serum values which are:

Total protein	6 to 8 g per 100 ml
Albumin	3.6 to 5.6 g per 100 ml
Globulin	1.3 to 3.2 g per 100 ml
A G ratio	1.5:1 to 2.5:1

There is considerable confusion in terminology as to exactly what constitutes globulin. As techniques of electrophoretic separation have become refined, more specific fractions are being resolved and arbitrarily identified as albumin components or globulin components, depending upon their rate or migration in an electrical field. It becomes difficult, if not impossible, to transfer electrophoresis terminology to protein fractions separated by less refined techniques. One such technique, called "salting out" is explained in the following paragraph with the understanding that a perfect parallel does not exist between fractions separated by this method and albumin or globulin fractions identified by other techniques. However, the correlation is sufficiently close to permit meaningful interpretation of the results.

In "salting out," advantage is taken of the fact that certain proteins are not soluble at their isoelectric points. Upon the addition of a neutral salt, such as sodium sulfate (Na_2SO_4) or sodium sulfite (Na_2SO_3), certain proteins will precipitate from solution. As concentration of the salt varies, the fraction which is precipitated from solution will vary. It has been determined that upon addition to 26 percent Na_2SO_4 or a slightly higher concentration of sodium sulfite, a fraction identified as globulin will precipitate from an aqueous solution of serum proteins. Within the past few years the use of Na_2SO_3 has largely replaced Na_2SO_4 because sodium sulfite agrees better with electrophoretic patterns. AFM 160-49 suggests using 5.7 ml of 28.3 percent sodium sulfite solution which is overlayed with 0.3 ml of serum. The use of 28.3 percent sodium sulfite in the procedure rather than various concentrations of sodium sulfite employed in older methods, yields albumin and globulin more consistent with electropheretic measurements. Sodium sulfite has better buffering power and will remain in solution at room temperature while sodium sulfate will crystallize out.

After the globulins have been precipitated with the sulfite mixture, ether is added, to cause the globulin to form a mat, as pictured in figure 3-3. Albumin remains in solution. The solution containing albumin may be analyzed by the biuret method. The figure calculated as representing the albumin fraction is then subtracted from the total protein to calculate the globulin. Similar salting out techniques are used to identify other specific fractions. For example, an ammonium sulfate reagent can be used to identify the gamma globulin fraction, and 12.5 percent Na_2SO_3 used to separate fibrinogen.

Besides salt precipitation and electrophoresis, other methods are in use for the assay of protein. The classic Kjeldahl method measures protein nitrogen which is essentially an index of the amount of protein present.

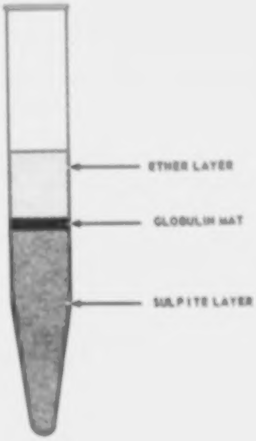


Figure 3-3. Diagram of tube in which globulins have been "salted out" from a serum-sulfite mixture.

The Kjeldahl method is frequently referred to in standardizing other methods. An entirely different concept involves the use of dyes to which proteins have specific affinities. One method reported used the dye-binding capacity of albumin with 2-(4-hydroxyazobenzene) benzoic acid (HABA). Later an ethylenedeamine tetracetate (EDTA) buffer was proposed to stabilize the dye reagent. This modification was applied to the Autoanalyzer as an automated method of albumin analysis.

Plasma Proteins Determinations. The blood proteins are of great importance in terms of clinical laboratory information because of their accessibility. The blood proteins may be separated in three groups: hemoglobin, red cell protein, and plasma protein. Fibrinogen, being a very large protein, constitutes normally about 5 percent of plasma proteins.

Fibrinogen. Normal human plasma contains 0.15 to 0.30 g-% fibrinogen, which is the important protein element that makes up a fibrin clot. The estimation of fibrinogen is a routine test performed to investigate clotting disorders. Its value is not limited to rare blood dyscrasias, because fibrinogen deficiencies frequently occur in conditions such as complications of pregnancy involving severe bleeding. Various techniques are available for the assay or estimation of fibrinogen, some of which are available as "kits" from commercial sources. In one method, 1 ml of freshly drawn blood is mixed with 0.1 ml topical thrombin (1000 NIH units per ml). The degree of clot formation is an index of the fibrinogen content. One entire category of fibrinogen tests depends upon the recovery of fibrin, the amount of which is dependent upon the amount of both fibrinogen and prothrombin. The use of a neutral salt (12.5 percent Na_2SO_3) is another means of assaying plasma for fibrinogen similar to the differential precipitation of globulin. Methods for quantifying fibrinogen on the basis of heat lability or electrophoretic mobility are largely obsolete.

Hemoglobin. Most of the protein in blood is in the form of hemoglobin. The molecular weight of hemoglobin is 64,500 amu. It is composed of about 10,000 atoms. Four of these atoms are iron, each capable of combining with two atoms of oxygen. Each atom of iron in the hemoglobin molecule is surrounded by a group of atoms which form the pigment called *heme*. The heme groups, in turn, are surrounded by a chain of amino acids. There are four amino acid chains which constitute the protein or globin parts of the molecule. Globin is a histone which yields histine upon hydrolysis. In all four chains there is a total of 574 amino acids in the hemoglobin molecule. It should be kept in mind that hemoglobin not only carries oxygen but also transports carbon dioxide to the lungs. Carbon dioxide which is carried by the cell is bound to the globin portion of the molecule. The subject of hemoglobin is covered in the course material on hematology, and is merely mentioned here as an important blood protein.

Exercises (219):

1. What is the best method for serum protein fractions determination?

2. What are some clinical importance for determination of A/G ratio?

3. Why is sodium sulfite recommended over sodium sulfate for the precipitation of serum globulins?

4. Why is ether added to the serum-sulfate mixture?

5. What reagent can be used to identify the gamma globulin fraction?

6. What reagent can be used to separate fibrinogen?

7. In simple terms, how does the classic Kjeldahl method measure protein?

8. What modification for albumin has been applied to the Autoanalyzer as an automated method for albumin analysis?

9. In what three groups can blood proteins be separated?

10. Approximately what percent of plasma proteins is fibronogen?

11. What are the normal values for fibrinogen in human plasma?

12. Most of the protein in the blood is in what form?

CHAPTER 4

Carbohydrates

IF ONE HAD TO choose the most indispensable chemical determination performed in the clinical laboratory, the choice would probably be the test for blood glucose. Laboratories that perform relatively few chemical determinations will sooner or later perform a test for glucose. Larger laboratories are likely to perform not only many glucose determinations but other carbohydrate studies chemically related to glucose. This might include lactose, xylose, or protein-bound carbohydrates, to mention just a few tests that may be done in the clinical laboratory.

In addition to prominence and wide application, carbohydrate analyses rank among the first body chemistries performed in the laboratory. Consequently, the field of carbohydrate chemistry is reasonably well developed and involves many different approaches and contributions over the years. A comprehensive review of the carbohydrate chemistry would require many volumes, even if limited to clinical application. This chapter will be limited to a brief discussion of carbohydrate chemistry and the most commonly performed tests for glucose. You may refer to a current index of technical and medical literature for an insight into the wide application of other carbohydrate tests which are, in many instances, of great clinical value. While many of these can be performed in the average laboratory, they are not included in the following limited discussion.

4-1. Chemistry and Physiology of Carbohydrates

Carbohydrates, or saccharides as they are commonly known, are of wide interest in clinical medicine. Glucose, a monosaccharide with the formula $C_6H_{12}O_6$, is of particular interest to the clinical laboratory. It is in the form of monosaccharides that carbohydrates are absorbed into the blood. Various deficits in absorption, metabolism, or in the regulatory mechanisms (such as endocrine disorders) are diagnosed and treated on the basis of the blood glucose level. For example, a deficiency of insulin, which is produced by the pancreas, causes diabetes mellitus. Carbohydrates occur in various forms, known as isomers, which will be explained at some length in this section, because isomerism is a fundamental aspect of carbohydrate chemistry. Information presented in this chapter will also be of benefit to you in your study of bacteriology by enabling you to better understand carbohydrate fermentations.

220. Indicate the definition, differences, chemical structures, and metabolism of the various given carbohydrates.

Classification of Carbohydrates. Glucose belongs to the chemical family of carbon-containing compounds known as *saccharides* or *saccharoses,* which are represented by the formula of $C_xH_{2y}O_y$. They do not contain atoms of other elements. From this formula it is apparent that saccharides contain hydrogen and oxygen in the same 2:1 ratio as they occur in water. Hence, the older term "carbohydrate," rather than "saccharide," is often used. The saccharides are classified according to structure. *Monosaccharides* have the simplest structure with the formula $C_xH_{2y}O_y$. They do not hydrolyze into other sugars, but remain unchanged. Glucose is an example of a monosaccharide with the formula $C_6H_{12}O_6$. *Disaccharides,* with the formula $C_n(H_2O)_{n-1}$, yield two simple sugars of the same or different type upon hydrolysis. Some examples of the disaccharide group are sucrose, lactose, and maltose, all with the formula $C_{12}H_{22}O_{11}$, as represented graphically in figure 4-1. Sugars with more than two saccharide groups are referred to as polysaccharides, some of which can be designated as $(C_6H_{10}O_5)n$. If n is less than 10, the term "oligosaccharide" is sometimes used. Starch, dextrin, cellulose, and glycogen are all examples of polysaccharides. They are less like common monosaccharides and disaccharides, in that higher molecular weight saccharides are not sweet, are less soluble, and form colloidal solutions which cannot be dialized.

Within each of the three major categories (that is, mono-, di-, and polysaccharides), carbohydrates are classified according to the number of carbon atoms each component sugar possesses. As indicated by the formula $C_6H_{12}O_6$, the monosaccharides of greatest biological interest have 6 oxygen atoms. Accordingly, they are classified as hexoses. Glucose, the hexose most common to the clinical chemist, is also called dextrose. You should not confuse dextrose with dextrin, which is

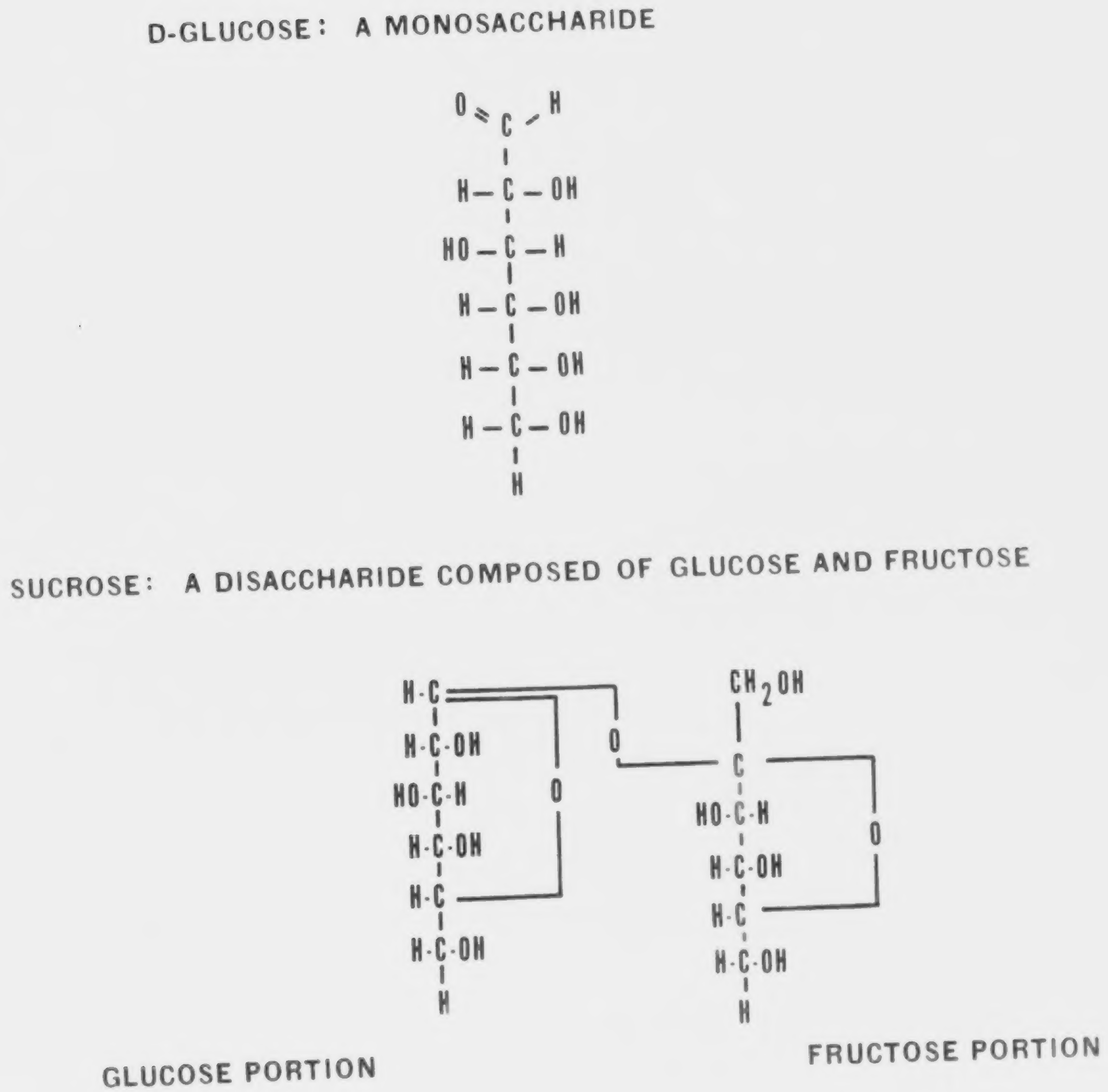

D-GLUCOSE: A MONOSACCHARIDE
SUCROSE: A DISACCHARIDE COMPOSED OF GLUCOSE AND FRUCTOSE
GLUCOSE PORTION
FRUCTOSE PORTION

Figure 4-1. Structural formulas of a monosaccharide, D-(+) glucose, and a disaccharide sucrose.

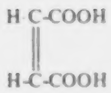
a polymer from partially hydrolyzed starch. Starch, cellulose, glycogen, and extrin are collectively classified as dextran and are all polysaccharides. But other monosaccharides besides the hexoses are known to the organic chemist and are of less interest to the clinical chemist. For this reason, the general formula for monosaccharides, $C_nH_{2n}O_n$, was given rather than $C_6H_{12}H_6$, which is limited to hexoses. Table 4-1 illustrates how some carbohydrates are classified.

Stereoisomerism. Carbohydrates occur as isomers. Isomers are forms of a substance which differ in atomic arrangement. The phenomenon of isomer occurrence is termed "isomerism." Stereoisomerism is due to spacial rather than structural arrangement, and occurs in two common forms: geometric and optical. Geometrical isomers are formed because some factor (as a double bond) prevents free rotation of the atoms within the molecule. The isomers formed by geometric isomerism are termed "cis-" and "trans-" and the difference is shown below in the diagrams of two geometric forms of butenedioic acid:

TABLE 4-1

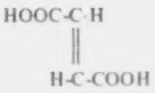
CLASSIFICATION OF CARBOHYDRATES (INCOMPLETE LISTING)

- **Carbohydrates**
  - Sugars
    - Monosaccharides
      - Dioses ($C_2H_4O_2$)
      - Trioses ($C_3H_6O_3$) -glycerose
      - Tetroses ($C_4H_8O_4$) -erythrose
      - Pentoses ($C_5H_{10}O_5$) -arabinose
      - Hexoses ($C_6H_{12}O_6$) -glucose
      - Heptoses, Octoses, etc.
    - Disaccharides
      - Sucrose, lactose, maltose, cellobiose, etc. ($C_{12}H_{22}O_{11}$)
      - Glucoxylose, etc. ($C_{11}H_{20}O_{10}$)
  - **Nonsugar**
    - Trisaccharides ($C_{18}H_{32}O_{16}$) -raffinose
    - Tetrasaccharides ($C_{24}H_{42}O_{21}$) -stachyose
    - Polysaccharides $(C_6H_{10}O_5)_{\underline{n}}$
      - **Dextran (dextrins, glycogen, starch, cellulose)**
      - **Galactosans, etc.**



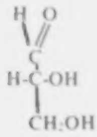
$$\begin{array}{l}H\text{-}C\text{-}COOH\\ \;\;\|\\ H\text{-}C\text{-}COOH\end{array}$$

cis-form



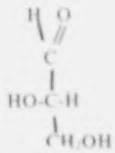
$$\begin{array}{r}HOOC\text{-}C\text{-}H\\ \|\;\;\\ H\text{-}C\text{-}COOH\end{array}$$

trans-form



$$\begin{array}{c}H\;\;O\\ \backslash\!/\!/\\ C\\ |\\ H\text{-}C\text{-}OH\\ |\\ CH_2OH\end{array}$$

(d) glyceraldehyde



$$\begin{array}{c}H\;\;O\\ \backslash\!/\!/\\ C\\ |\\ HO\text{-}C\text{-}H\\ |\\ CH_2OH\end{array}$$

(l) glyceraldehyde

The double bond illustrated prevents the atoms from rotating; thus, there are two distinctly different geometric possibilities.

An asymmetric atom (in our study it will always be a carbon atom) is one which has a different atom or radical attached to each of its four valence bonds. Glyceraldehyde, in the formula below, has one asymmetric carbon atom. This carbon has attached to it a hydrogen, a hydroxyl group, an aldehyde group, and an alcohol group. The isomers formed in optical isomerism are mirror images of each other. Looking again at glyceraldehyde, we see that there are two optical isomers, each a mirror image of the other. Such isomers are called enantiomorphs (from the Greek meaning "opposite form").

In naming these compounds, the position of the OH group nearest the end opposite the carbonyl group ($C = O$) determines the (d) or (l) form, as shown above with glyceraldehyde.

In early studies it was erroneously believed that the two variations in spacial configuration always correlate with optical rotation. Optical isomers differ in their ability to rotate polarized light due to asymmetrical or crystalline structure. Polarized light is defined as light which vibrates in only one plane. It is produced as illustrated in figure 4-2. When polarized light is passed through a solution of an optically active substance, the plane of polarization is diverted either

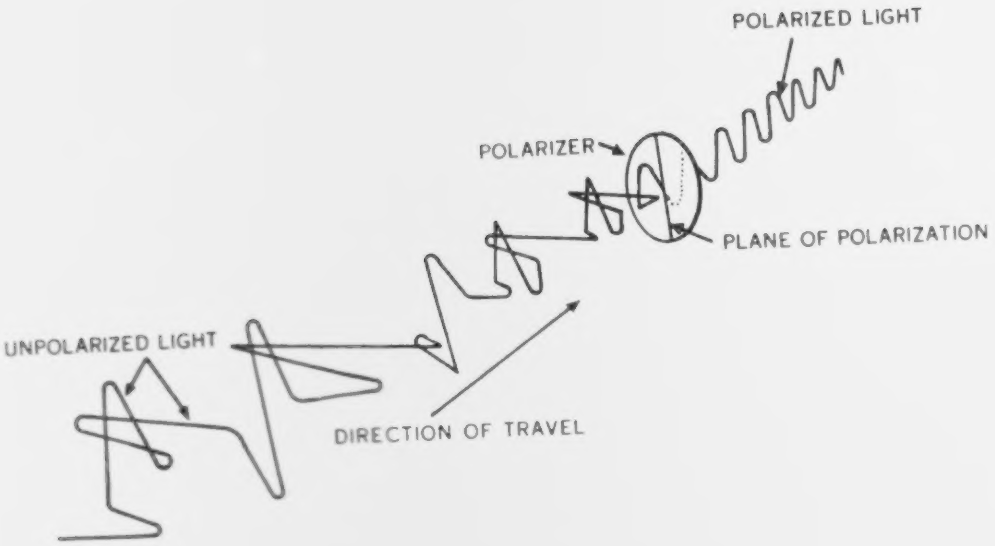
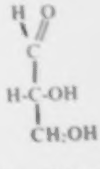


Figure 4-2. Diagram illustrating principle of polarization.

to the right or to the left. Thus, for each carbohydrate there would theoretically exist two optical isomers based on molecular configuration. Compounds which conform to the (d) structure when in solution were believed to be those which rotate polarized light to the right, and were called dextrorotatory. Those which, based on their structure, were thought to rotate polarized light to the left were known as levorotatory or the (l) form. A mixture of 50 percent each of the (d) and (l) isomers produces no rotation and is known as a racemic mixture.

As pointed out, optical properties do not show a clear correlation with spacial configuration. Many optically active compounds do not rotate polarized light as predicted according to structure. Rather, some of the (d) forms rotate polarized light to the left. As a result, a second system of nomenclature became necessary to describe the effect on polarized light. Refer again to the structure of (d) glyceraldehyde.



(d) glyceraldehyde

Under the second system, the compound (d) glyceraldehyde becomes d(+) glyceraldehyde, because it has the (d) structure and rotates polarized light to the right as indicated by a plus sign. If this compound were to rotate the light to the left, it would be called the d(−) glyceraldehyde.

Each asymmetric carbon atom can yield two enantiomorphs, and as the number of asymmetric carbons increases, the number of isomers increases. The possible number of enantiomorphs for a compound is 2^x, in which the x stands for the number of asymmetrical carbons. The biological activity of carbohydrates depends upon the symmetrical arrangement of the carbon atoms. For this reason, you should understand the meaning of symmetry and its relationship to isomerism. Optical properties, particularly with reference to polarized light, provide a means of studying and distinguishing between the various isomers.

Exercises (220):

Match each of the following column B items with the statement in column A. Each column B item may be used once or more than once.

Column A

___ 1. Chemical family of carbon-containing compound represented by the formula $C_xH_{2y}O_y$.
___ 2. Are classified according to structure.
___ 3. $C_n(H_2O)_n$ = ..
___ 4. Sugar with more than two saccharide groups, some designated as $(C_6H_{10}O_5)_n$.
___ 5. Starch, dextrin, cellulose and glucose.

Column B

a. Carbohydrates.
b. Glucose.
c. Saccharides or saccharoses.
d. Disaccharides.
e. Monosaccharides.
f. Polysaccharides.
g. Dextrose.
h. Polarized light.
i. Optical isomers.
j. Isomers.
k. "Cis" and "trans."
l. (d) − isomer.
m. (d) + isomer.

Column A

___ 6. Within each of the three major categories, they are classified according to the number of carbon atoms each component sugar possesses.
___ 7. The hexose most common to the clinical chemist; also called dextrose.
___ 8. Should not be confused with dextrin, which is a polymer from partially hydrolyzed starch.
___ 9. General formula for this group.
___ 10. Forms of the same substance which differ in atomic arrangement.
___ 11. Meet the definition of isomers and are additionally characterized by their rotational effect on polarized light.
___ 12. Light energy which vibrates in only one plane.
___ 13. The isomers formed by geometric isomerism.
___ 14. This form of isomer rotates polarized light to the right.
___ 15. This form of isomer rotates polarized light to the left.

221. Identify the given processes in the digestion, absorption, and metabolism of carbohydrates.

Digestion and Absorption of Carbohydrates. Polysaccharides and disaccharides are broken down by enzymes into monosaccharides before absorption occurs. Maltase, sucrase, and lactase break down maltose, sucrose, and lactose, respectively, in the intestine. Salivary and pancreatic amylase change starch to maltose. Most of the monosaccharides absorption takes place in the small intestine at a fairly constant rate. The rate of absorption is also characteristic of the particular monosaccharide; and is, therefore, not entirely a matter of simple diffusion, but rather a selective process dependent upon the activity of the intestinal mucosa. The absorbed sugar is picked up by blood capillaries and transported via the portal vein to the liver. Within the liver, the blood carrying the sugar flows through a capillary network and the sugar is taken up by the liver cells.

Metabolism of Carbohydrates. Glucose is used in one of three ways: for energy, for storage after conversion to glycogen, and for conversion to other substances. In addition to glucose from carbohydrates absorbed in the diet, glycogen may be formed from glucose which has been synthesized in the liver from noncarbohydrate sources. The process of glucose formation from noncarbohydrates is called gluconeogenesis. Glucose may be converted to carbohydrates, such as ribose or noncarbohydrate substances, for example, amino acids.

Let's take a closer look at the processes involved in the use of glucose for energy. For convenience, let's describe the breakdown of glucose as aerobic and anaerobic. In aerobic metabolism, glycogen (or glucose) is carried through a series of carbohydrate-phosphate complexes to pyruvic acid, which then oxidizes to carbon dioxide and water. The series of steps involved in the aerobic metabolism of glucose is called the Krebs cycle, tricarboxylic acid (TCA) cycle, or citric acid cycle. In the anaerobic metabolism of glucose (glycolysis), pyruvic acid is reduced to lactic acid. Certain other pathways are available for the breakdown of glucose, including the so-called pentose shunt in which a ribose-5-phosphate or ribulose-5-phosphate compound is formed intermediate to certain other steps and the Krebs cycle. It should be pointed out that metabolism of glucose is not limited the liver. Muscle plays an active role in carbohydrate metabolism. Both the formation of glycogen (glycogenesis) and the breakdown of glycogen (also called glycolysis) take place in the muscle in a manner similar to reactions which take place in the liver. The obvious purpose for muscle glycolysis is to supply energy to the muscle.

Exercises (221):

Match each of the following column B items with the statement in column A. Each column B item may be used once, more than once, or not at all.

Column A

___ 1. Form into which polysaccharides and disaccharides are broken by enzymes before absorption occurs.
___ 2. Organ in which most of the absorption of monosaccharides takes place.
___ 3. For energy, for storage after conversion to glycogen, and conversion to other substances.
___ 4. The process of glucose formation from noncarbohydrates.
___ 5. Glycogen is carried through a series of carbohydrate phosphate complexes to pyruvic acid which then oxidizes to carbon dioxide and water.
___ 6. Series called the Krebs cycle.
___ 7. Glucose, pyruvic acid is reduced to lactic acid.
___ 8. Organ in which glycogenesis and glycolysis takes place.

Column B

a. Usage of glucose.
b. Small intestine.
c. Large intestine.
d. Kidneys.
e. Monosaccharides.
f. Gluconeogenesis.
g. Aerobic metabolism of glucose.
h. Anaerobic metabolism of glucose.
i. Muscle.

222. Identify the factors responsible for maintenance of blood glucose levels.

Blood Glucose Levels. The normal fasting glucose level of the blood is 70 to 100 mg-%. This level is often referred to as the true glucose level to distinguish it from figures which include nonglucose reducing substances. The Folin-Wu procedure which produces normal fasting glucose levels of 80 to 120 mg-% includes other reducing substances. Since the blood glucose levels depend upon both the rate at which glucose enters the blood and the rate of glucose removal, levels depend upon various factors. These factors are insulin, epinephrine, pituitary hormones, and thyroxin.

Insulin. This hormone is secreted by the pancreas, and regulates both the rate of glucose output from the liver and the rate of glucose utilization. It also regulates the transfer of glucose across cell membranes. An excess of insulin, known as *hyperinsulinism,* causes a decrease in blood glucose levels (hypoglycemia). *Diabetes mellitus* is a metabolic disorder resulting from a lesion of the pancreas or overactivity of the pituitary and adrenal glands, which has a direct effect on the pancreas. It is characterized by a lack of insulin, for example, *hypoinsulinism.* Glucose often appears in the urine, and blood sugar levels are abnormally high (hyperglycemia) in patients with diabetes mellitus.

Epinephrine. Produced by the adrenal gland, epinephrine regulates the rate of glucogen breakdown in the liver. Epinephrine is also called adrenalin. In adrenal cortical insufficiency the blood sugar level is frequently below normal.

Pituitary hormones. Hormones from the pituitary gland, which is attached to the base of the brain, retard the use of glucose in the body; hence, their presence tends to raise blood glucose levels. A head injury or tumor of the pituitary may produce *diabetes insipidus.* In this condition the blood sugar is not necessarily elevated and there is no sugar in the urine, but urine is excreted in copious amounts and has a low specific gravity. The term "diabetes" means an excessive discharge of urine, from the Greek words for "siphon through." The effect of anterior pituitary insufficiency is essentially the same as adrenal insufficiency.

Thyroxin. Regarded as the principal active component of the thyroid hormone, thyroxin increases the breakdown of glycogen in the liver. Hyperthyroidism results in a decreased tolerance for glucose. The process of glucose production and the use of glucose are in dynamic equilibrium to maintain a fairly constant level of glucose in the blood. The regulatory mechanisms are such that a predictable curve can be established to depict the rise and fall in blood sugar levels following the ingestion of glucose. Known as a glucose tolerance curve, a graphic representation of glucose levels at intervals after an intake of glucose of other carbohydrates has great diagnostic significance. In a normal response to glucose intake, the fasting specimen should be within the normal limits, 80-110 mg/dl. The 1/2-hour level should exceed the fasting level by less than 75 mg/dl. Further, the 1-hour level should be less than 180 mg/dl; the 2-hour less than 140 mg/dl and return nearly to a fasting level at the end of the third hour. All urine specimens collected during the test should be negative for glucose. Three different glucose tolerance curves are shown in figure 4-3.

Various abnormal glucose tolerance curves and their clinical importance are matters of diagnostic concern more related to the interpretation of laboratory results than to the performance of them. Besides occurring in diabetes mellitus, increased glucose levels may be found in uremia, nephritis, hyperthyroidism, during pregnancy, and in infectious states. As previously mentioned, low blood glucose levels may be found in hypothyroidism, hepatic disease, Addison's disease, and hyperinsulinism. Some individuals have a low renal threshold for glucose; and hence, glucose may appear in the urine (glycosuria) in the absence of a pathological condition. This condition is frequently described as renal diabetes.

Exercises (222):

Match the following by placing the letter of the column B item beside the number of the column A item or items that most nearly describes it. Each element in column B may be used once or more than once.

Column A

___ 1. Regulates the rate of glycogen breakdown in the liver.
___ 2. Regulates both the rate of glucose output from the liver and the rate of glucose utilization.
___ 3. Regulates transfer of glucose across the cell membranes.
___ 4. Metabolic disorder resulting from a lesion of the pancreas or over activity of the pituitary and adrenal glands.
___ 5. Retards the use of glucose in the body.
___ 6. Blood sugar may be normal, no sugar in the urine. Urine excreted in large amounts with low specific gravity.
___ 7. Increases the breakdown of glycogen in the liver.
___ 8. Condition characterized by glycosuria due to a low renal threshold for glucose.

Column B

a. Insulin.
b. Epinephrine.
c. Pituitary hormone.
d. Diabetes insipidus.
e. Diabetes mellitus.
f. Thyroxin.
g. Renal diabetes.
h. Normal response to glucose intake.
i. Abnormal response to glucose intake.

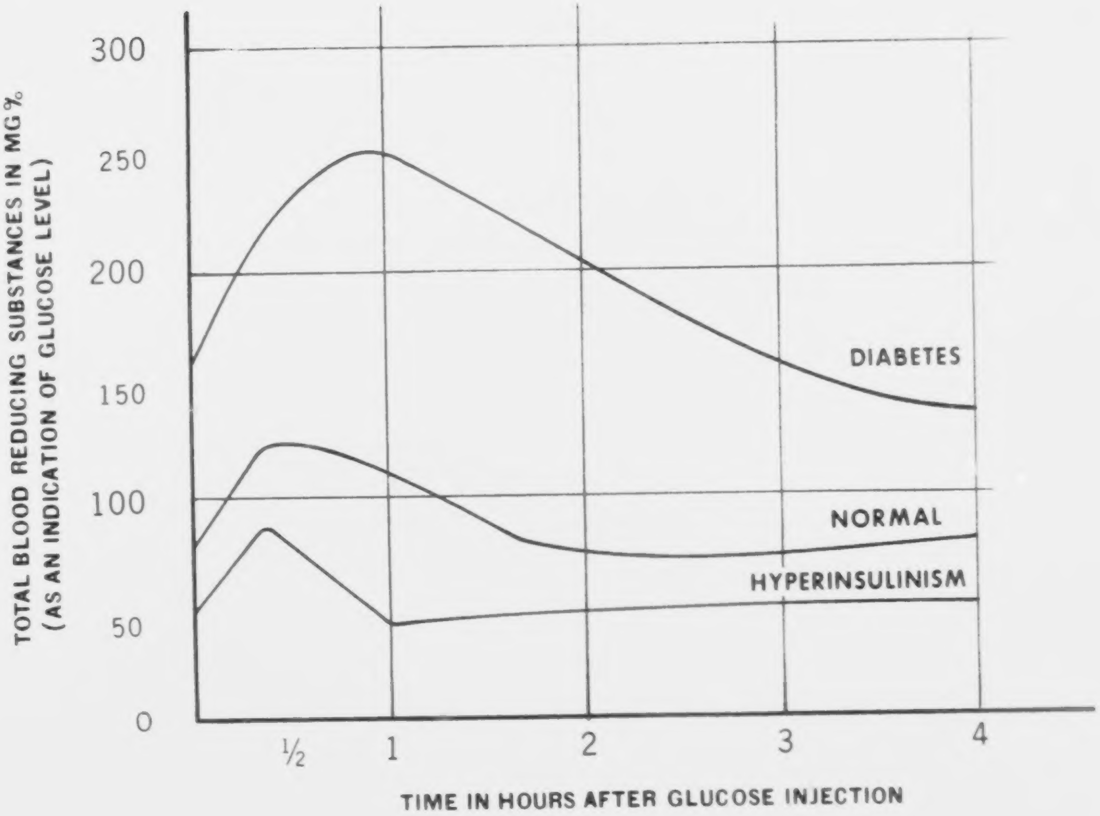


Figure 4-3. Glucose tolerance curves.

Column A

___ 9. Fasting specimen within normal limits; 1-hour specimen less than 180 mg/dl; 2-hour specimen less than 140 mg/dl and all collected urine specimen negative.

___ 10. Fasting specimen within normal limits; 1-hour specimen greater than 180 mg/dl; 2-hour specimen less than 140 mg/dl and all collected urine specimen negative.

4-2. Laboratory Tests for Glucose

As a laboratory technician, your most immediate concern is performance of laboratory tests. This section outlines various tests for glucose with a brief analysis of their relative merits.

223. Identify the methods for glucose determination, reagents used in the procedures, and possible sources of errors.

Methods for Glucose Determination. The search for simpler and more specific methods for the determination of glucose is continuous. Many of these consist of automatic versions of manual methods while others involve different approaches or modifications. Glucose methods are considered to fall into two categories: chemical and enzymatic methods. We will discuss briefly the various approaches to these methods.

Folin-Wu procedure. In the Folin-Wu method, a protein-free filtrate is heated with an alkaline copper solution. The reduction of cupric hydroxide produces cuprous oxide. Phosphomolybdic acid is then reduced to molybdenum blue, which has an uncertain chemical composition. This blue color is compared with that of a glucose standard which is available through medical supply channels. If used with a tungstate filtrate, the Folin-Wu method does not indicate the true glucose level of blood. Besides other reducing sugars, the procedure will detect glutathione, uric acid, ascrobic acid, and some amino acids. If used with a zinc (Somogyi) filtrate, the Folin-Wu procedure is quite specific for glucose. Many, if not most, laboratories have abandoned this procedure for methods more specific for glucose. This is a wise decision, since the error due to nonglucose reducing substances can be substantial.

Ortho-toluidine procedure. In principle, color development is based on the reaction of glucose in the specimen with ortho-toluidine in glacial acetic acid. A green glycosylamine is formed, the amount of color being preparational to the concentration of glucose present. Described in some detail by Dr. K.M. Dubowski in the journal "Clinical Chemistry," June 1962, this method is similar in principle to a rapid method for aldosaccharide determinations, described by Dr. Eric Hultman in "Nature," January 1959. The method considered Dubowski, modified, is outlined in AFM 160-49. Ortho-toluidine, as shown in figure 4-4, is only one of many aromatic amines which yields a color complex with glucose.

Of the nonenzymatic techniques, the o-toluidine method is rapidly becoming most widely used because of its simplicity, high degree of specificity, and applicability to serum without deproteinization.

Since the reaction is not affected by the reducing properties of glucose, only a small quantity of physiologically occurring compounds react with o-toluidine to yield substances absorbing at the wavelength used to measure the colored compound formed with glucose. Azotemia (bilirubin up to 20 mg/100 ml), which commonly causes erroneous high glucose values with copper reduction methods, has no effect on the o-toluidine methods.

A more stable o-toluidine reagent is now available than that used with earlier methods. Thiourea is used as a stabilizing agent, and some other difficulties are minimized because a semimicro amount is used. The color of the cooled reaction mixture is stable up to 30 minutes and then the color slowly decreases.

The color development follows Beer-Lambert law up to a glucose concentration of 250 mg per dl. Repeat glucose concentrations higher than 250 mg per dl on diluted serum specimen or double the quantity of o-toluidine reagents used and multiply the final reading by two. Values obtained by this method are comparable to those obtained by other "ture" glucose methods. The precision of the method is ±5 percent for glucose values between 50 and 300 mg/100 ml.

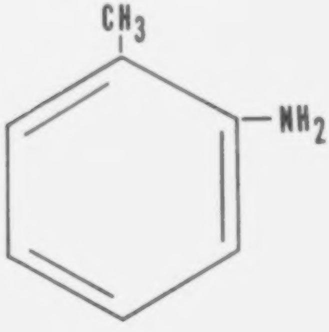


Figure 4-4. Structural formula of ortho-toluidine (2-aminotoluence).

Enzymatic procedures. The use of enzymes in order to achieve ultimate specificity initially involves the use of yeast, by obtaining the difference between reducing sugars before and after yeast fermentation. Yeast had been widely used in this connection until the advent of purified enzymes. Greater specificity was obtained by the use of a single purified enzyme which acts on glucose but not other physiologically occurring carbohydrates or derivatives. One such purified enzyme is glucose oxidase, which is specific for β-D-glucose. It converts glucose to gluconic acid and hydrogen peroxide. The hydrogen peroxide is used as the key to color development in most enzyme procedures by reacting it with a compound such as o-dianisidine to form a color. The color reaction may be diagrammed as follows:

$$\text{Glucose} + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{dye} - H \xrightarrow{\text{peroxidase}} \text{Color} + H_2O$$

Glucose oxidase is specific for one isomer of glucose (beta-D-glucose), and it may, therefore, be necessary to incorporate another enzyme in the reaction which induces a shift from the nonutilizable alpha isomer present to the beta form which can be oxidized. The shift is termed "mutarotation," and is essential to insure complete assay of the glucose. About 35 percent of blood glucose is in the nonusable, or alpha, form. A procedure for this method is outlined in AFM 160-49. Sources of error include (a) variability of results when using whole blood samples, due to a normal goycolytic system present in whole blood and the enzymes inhibitors present in serum plasma or whole blood, and (b) rubber tubing used for dispensing water has occasionally inhibited color development and should not be used. The advantages of the method are its specificity, its sensitivity, and elimination of the need for a filtrate.

Dextrostix, a paper strip impregnated with a glucose oxidase peroxidase-chromogen system, is used for semiquantitative estimates of blood glucose levels. One enzymatic procedure for glucoses uses hexokenase and glucose 6-phosphate dehydrogenase to convert glucose to 6-phosphate gluconate. Further, the principle has been applied to the Autoanalyzer and other automatic equipment

Somogyi-Nelson Ture Glucose. Alkaline copper solution is heated with a filtrate of the specimen which causes cupric hydroxide to be reduced to cuprous oxide. Arsenomolybdate is then added and reduced to form a complex which is a deep green-blue color. This color is proportional to the amount of reducing sugar present. A Somogyi filtrate consists of 1 part blood, 5 parts water, 2 parts 0.3N barium hydroxide, and 2 parts 5 percent zinc sulfate ($ZnSO_4 \cdot 7H_2O$). The use of a zinc filtrate may interfere with copper reduction when micro procedures are used.

Ferricyanide Reaction. Several ferricyanide methods have been used to determine glucose. The reactions depend upon the reduction in an alkaline solution of yellow ferricyanide ions, $Fe(CN)_6^{---}$, to colorless ferrocyanide ions, $Fe(CN)_6^{----}$, by glucose. This technique has gained interest since its institution as an automated method by the use of an Autoanalyzer. A serum dialysate is mixed with alkaline ferricyanide in a continuous stream. It is then sent through a glass delay coil maintained at 95° C. The ferricyanide is reduced to a relatively colorless ferrocyanide dependent upon glucose concentration. The reacted stream goes through a flow cuvette, where the loss in color is read spectrophotometrically and recorded before the stream goes to waste. This Autoanalyzer procedure is outlined in figure 4-5.

Several aspects of automation may need clarification at this point. A dialysate is the material which diffuses (or dialyzes) through a membrane. In this glucose method, dialysis is used to render the specimen protein-free. This process occurs at a constant rate as the diluted serum sample (specimen stream) is pumped through the dialyzer module on one side of the membrane. You can see from figure 4-5 that alkaline ferricyanide reagent (reagent stream) also passes through on the opposite side of the dialyzing membrane from the sample and carries sample dialysate along with it. The specimen stream goes to waste from the dialyzer. The reagent stream, containing dialysate, continues on to the 95° C heating bath where reduction of ferricyanide occurs. Transport tubing carries the reacted stream to a colorimeter where the stream is debubbled before passing into a "flow-through" cuvette. The optical density is automatically recorded on a graph paper strip. This complete process will quantitate 60 samples per hour. Both glucose control serum and standards are included in each run for standardization and quality control. Other concepts involving automatied analytical methods will be discussed as they develop in this text.

Exercises (223):

Match the methods listed in column B with the sources of errors and reagents to which they closely relate in column A by placing the letter of the column B item

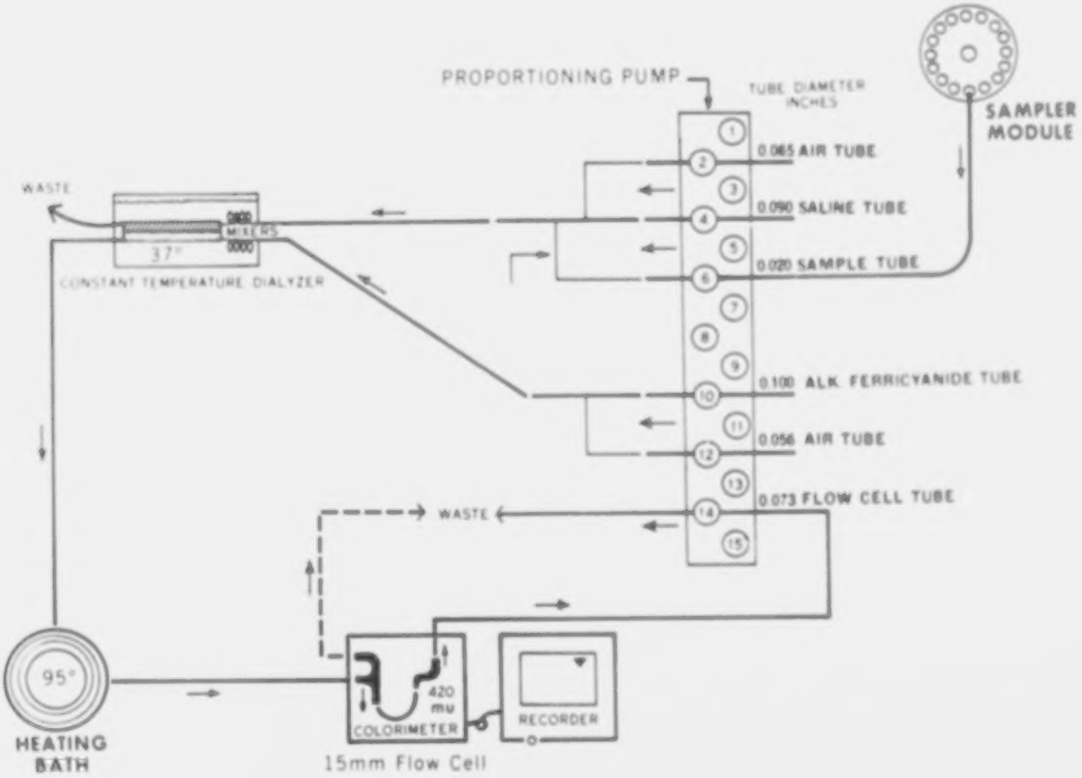

PROPORTIONING PUMP
TUBE DIAMETER INCHES
SAMPLER MODULE
1
2
0.065 AIR TUBE
3
4
0.090 SALINE TUBE
5
6
0.020 SAMPLE TUBE
7
8
9
10
0.100 ALK. FERRICYANIDE TUBE
11
12
0.056 AIR TUBE
13
14
0.073 FLOW CELL TUBE
15
WASTE
MIXERS
37°
CONSTANT TEMPERATURE DIALYZER
WASTE
95°
HEATING BATH
420 mu
COLORIMETER
RECORDER
15mm Flow Cell

Figure 4-5. Ferricyanide method for glucose.

beside the number of the column A item. Each column B item may be used once or more than once.

Column A

___ 1. Dialysis is used to render specimens free of protein in this procedure.

___ 2. Will detect glutathione, uric acid, ascorbic acid, and some amino acids.

___ 3. Use a filtrate of 1 part blood, 5 parts water, 2 parts 0.3N barium hydroxide, and 2 parts 5 percent zinc sulfate.

___ 4. A green glycosylamine is formed proportional to the concentration of glucose.

___ 5. Is specific for β D-glucose and converts glucose to gluconic acid and hydrogen peroxide.

___ 6. Phosphomolybdic acid is reduced to molybdenum blue.

___ 7. Inhibitors present in plasma or whole blood affect results in this procedure.

___ 8. Color development follows Beer-Lambert law up to a glucose concentration of 250 mg per dl.

___ 9. Color development can be inhibited by rubber tubing used for dispensing water.

___ 10. Thiourea is used as a stabilizing agent.

___ 11. As a color developer hydrogen peroxide is reacted to o-dianisidine to form a color.

___ 12. Color reaction is stable up to 30 minutes and then slowly decreases.

Column B

a. Folin-Wu procedure.
b. Ortho-toluidine procedure.
c. Enzymatic procedure.
d. Somogyi-Nelson true glucose.
e. Ferricyanide procedure.

224. Name two methods for glucose determinations, and cite procedures, terms, and advantages and disadvantages of the two methods.

Other Glucose Procedures. Electrophoresis and paper chromatography are both used for the determination of glucose, although neither will be further discussed in this course in connection with glucose. Various reduction reactions besides those of copper and ferricyanide are also used, including the reduction of bismuth and the reduction of potassium permanganate. One reaction which is specific for sugars uses phenol in a solution of aqueous methyl salicylate. This is the so-called PMS reagent available commercially. Protein, particularly albumin, interferes with the reaction, elevating the results. A small amount of serum, urine, or spinal fluid is generally used rather than whole blood. Chemically, PMS reagent functions through combination of the phenolic hydroxyl group with the aldehydic ending of the sugar molecule. There are also many other glucose procedures which are not discussed here. It might be added that with most methods for determining sugar, deproteinization is responsible for the greatest difference in values obtained.

Glucose Tolerance Tests. In the usual glucose tolerance procedure, a measured amount of glucose is administered orally, and at periodic intervals blood and urine samples are analyzed for glucose and ketones. The amount of glucose administered is generally 50 to 100 gm for an adult. There is no evidence to suggest that the amount of glucose administered must be exact as long as uniformity is maintained. Glucose, which is available through supply channels, can be weighed on a laboratory balance. Flavored commercial preparations may be used in place of those available through supply channels.

A report on the Standardization of the Oral Glucose Tolerance Test by the American Diabetes Association Committee on Statistics provides excellent guidelines. It is suggested that the patient ingest at least 150 gm of carbohydrate daily for three days or more prior to the test. A glucose tolerance test should not be done routinely during hospitalization of an acutely ill patient whose dietary intake has been decreased. Guidelines for interpretation are based on values obtained int he ambulatory patient. Hence, the test should be performed with greater validity after the patient has resumed normal activity.

The test begins with a fasting blood sample, and the patient should not eat during the test. Further, vigorous exercise should be avoided, since this reduces the blood glucose level. A patient may become restless during the hourly intervals and use this time to rush about the base or do other physical work. He or she should be cautioned to refrain from such activity. There is no objection to the patient drinking moderate amounts of water during the test if desired.

Some authorities believe that an oral glucose tolerance test leaves much to be desired and should be replaced by the IV (intravenous) tolerance test. This would appear to be a matter of clinical judgment based on practical considerations and needs of the particular patient. In many cases a 2-hour postprandial specimen could be used to attain the medical objective, but again, this would be for the physician to decide. The intent of a 2-hour postprandial specimen is to measure the blood glucose level 2 hours after an adequate carbohydrate meal, usually to aid in the diagnosis of diabetes.

Since most tolerance tests are done in the morning to meet laboratory schedules, the meal may be less than adequate. Breakfast means different things to various people, ranging from dry toast to a three-course meal. Therefore, it is better to insure adequate intake by supplying 50 to 100 g of glucose. Further, it insures conformity to a schedule and reduces physical exercise of the patient, who might travel some distance between the laboratory and a place to eat. A fasting specimen followed by a 2-hour postprandial specimen is an expedient which has replaced many of the lengthy glucose tolerance tests performed in U.S. Air Force medical facilities and elsewhere. As noted in the preceding section, figure 4-3 shows the normal and a few abnormal responses to glucose intake. A detailed discussion of particular glucose tolerance curves, together with associated data, can be found in the medical literature, and is not usually a primary concern of the laboratory.

Exercises (224):

1. What other two general methods are available for glucose determination?

2. Among the methods for glucose determination, what step is responsible for the greatest difference in values obtained?

3. What is meant by a postprandial glucose test?

4. (a) List some general arguments for a glucose tolerance test as opposed to a 2-hour postprandial. (b) Cite some arguments against a glucose tolerance test as opposed to a postprandial blood sugar.

CHAPTER 5

Enzymes

ENZYMES catalyze chemical reactions which normally would not take place without their presence. Some reaction would take place however, but very slowly. They can be isolated, and afterwards be crystallized like other proteins, and are denatured by heat. They are inactivated by microorganisms and are sensitive to heavy metals, detergents, changes of pH, and ionic strength. By now about 1500 enzymes have been identified. This diversity of enzymes is explained by the fact that a specific enzyme is required for each step in the breakdown of cell nutrients.

Many investigators studied particular enzymes in their more or less natural cellular environment through the control of interfering or unrelated metabolic activities of the cell. Today, many of the enzyme studies in hospital laboratories are pursued along such biological lines through the study of enzyme systems and of mixtures of enzymes in serum, whole blood, etc. Because of its proven worth, the rapidly developing field of enzyme chemistry has taken over a significant share of clinical chemistry. As will be discussed more fully, enzymes control an almost infinite number of chemical reactions in the body. Yet, each enzyme is specific for the reaction it catalyzes and can, therefore, usually be related to a particular body function. Although many other chemistry procedures will always be essential, enzyme studies are gaining increasing attention and usefulness.

In this chapter we will attempt to answer two basic questions: (1) What are enzymes? and (2) How do they act? We will discuss the principles of the methods for measuring enzyme activity, and the possible factors responsible for abnormal values. Brief reference is made to enzyme determination by ultraviolet methods.

5-1. Enzyme Chemistry

Enzyme chemistry is the study of biocatalysts and is one of the most rapidly developing areas of clinical chemistry. In order to study enzymes in the laboratory, you must control all of the factors that affect their activity. These are listed for you. Because of the relatively low concentration of enzymes in body fluids, enzymes are reported in arbitrary units of activity under specified conditions. In reporting enzyme concentrations, you should always identify the type of units expressed. The units expressed are related overall to the nature of the specific enzyme.

225. Describe the measurement of enzyme activity by citing units of measurement, factors affecting activity, and methods of nomenclature.

Chemical Nature of Enzymes. Enzymes are all protein in nature and are produced by various cells of the body. They control all of the reactions that occur in the body. Because they control biochemical reactions, they are called biocatalysts. A catalyst is defined as an agent which controls the speed of a reaction without itself reacting or undergoing change. There is some recent evidence, however, that enzymes may undergo some changes of structure when they catalyze biochemical reactions. Described as feedback inhibition, this process is thought to be one in which the enzyme molecule undergoes a rearrangement of subunits. Further, each enzyme is thought to exist in two alternative structural forms, a reactive and a nonreactive state.

As you may recall from the discussion of protein chemistry, the structure of a protein molecule is very complex. This places limitations on the structural analysis of enzymes with methods available for detecting changes in molecular arrangement or special configurations. For our purposes it is less important to analyze theoretical mechanisms than to recognize behavioral properties. An awareness, for example, that enzymes are extremely effective in low concentrations causes the clinical chemist to seek a particular way of reporting their presence and effectiveness. Conventional expressions of concentration such as mg/dl would not prove to be a sensitive enough scale for the assay of enzymes. To overcome this problem, the biochemist has selected units of *activity*, and it is in terms of activity rather than concentration that enzymes are measured. Consequently, factors which affect enzyme activity must be known and controlled.

It has been recently proposed that all enzyme concentrations be expressed in terms of *international units* (IU). A unit is defined as 1 μM (micromole) of substrate used per minute per liter of serum under specified conditions of pH and temperature. Because the older units have been firmly established, these units have not come into general use in the clinical laboratory.

In many instances, the use of the international units would make for better comparison between methods.

For example, normal ranges for three alkaline phosphatase methods (Shinowara-Jones-Reinhart, King-Armstrong, and Bessey-Lowry-Brock) are given as 2.2–8.6, 3.7–13.1, and 0.8–2.9 units, respectively. These units appear to be all different, but when converted into international units, the ranges are essentially the same: 12.0–46.7, 13.0–46.0, and 13.3–48.3. You can see the importance for standardizing the reporting of enzymes values.

Factors Affecting Enzyme Activity. There are many factors that affect enzyme activity. Among these are the enzyme concentration, temperature, the pH of the solution, radiation, inhibitors, and time. In the next few paragraphs, we will discuss each of these factors.

Concentration. The concentration of an enzyme will determine the rate of activity, although a curve plotted as concentration versus activity will not necessarily be linear. That is, activity varies with enzyme concentration, but is not directly proportional under all conditions and tends to reach a plateau, as shown in figure 5-1. The very fact that an increase in concentration can be measured in the laboratory is useful, because the enzyme concentration in tissues and body fluids changes in certain clinical conditions. In most cases, it is the concentration of enzyme which is actually being measured when the technician expresses enzyme activity in arbitrary units. Likewise, concentration of the component upon which an enzyme acts to produce a chemical change will determine the activity expressed. The substance which undergoes the chemical change is called a *substrate*. Thus, we see that the concentration of substrate and the concentration of enzyme affect the activity of an enzyme.

The illustration in figure 5-2,A, depicts the process whereby E represents the enzyme, ES the enzyme-substrate complex, and P the product of the enzyme reaction. The "active site" of the molecule is the site at which the substrate is bound. In B of this figure, you may note that if the substrate concentration is low, then the enzyme is surrounded by only a small number of substrate molecules (1), and the chances that a molecule "hits" the binding site of E are fewer than with high concentrations of substrate. When the substrate concentration is increased and the reaction product released, the chances for ES to form increase and the time to fill the empty binding site becomes shorter as many substrate molecules surround the enzyme (2).

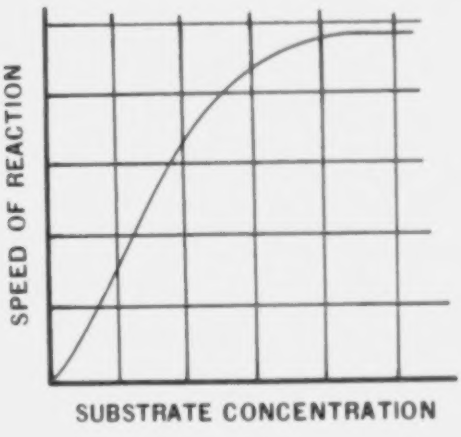


Figure 5-1. Effect of substrate concentration of enzyme activity.

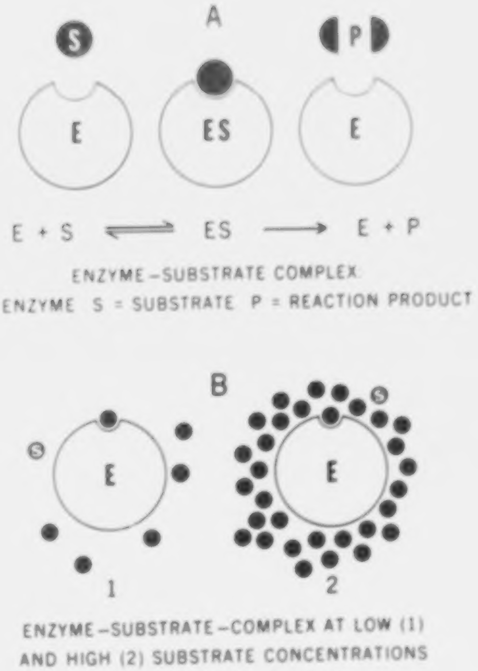

A
S
P
E
ES
E
E + S ⇌ ES ⟶ E + P
ENZYME–SUBSTRATE COMPLEX
E = ENZYME S = SUBSTRATE P = REACTION PRODUCT
B
S
E
E
1
2
ENZYME–SUBSTRATE–COMPLEX AT LOW (1)
AND HIGH (2) SUBSTRATE CONCENTRATIONS

Figure 5-2. Formation of the enzyme-substrate complex and enzyme-substrate at low and high concentrations.

Temperature. In general, the activity of any enzyme will approximately double for every 10° rise in temperature on the centigrade scale. Because enzymes are protein in nature, a temperature that is too high will inactivate the enzyme and even destroy it. Most human enzymes are denatured at 60° C. Consequently enzymes are described as heat labile. It is also true that a decrease in temperature will retard the activity of many enzymes. Most enzymes have very little activity at low temperatures (a useful fact for the technician who wishes to store a sample of serum or other body fluid containing enzymes). Ice formation may inactivate an enzyme unless quick-freeze methods with dry ice or lyophilization (fast dry freezing) is used. In most clinical laboratories if an enzyme serum specimen must be stored for a short period of time, 5° C is preferable to slow freezing. Of course, lyophilized specimens may be stored for many months. Some enzymes may continue to show significant activity at a decreased temperature. This is, incidentally, the primary reason why certain frozen foods should be treated with boiling water to inactivate the enzymes which cause them to deteriorate even at subzero temperatures. For the clinical laboratory worker, controlling temperatures is a constant concern. Enzyme studies are usually made in a water bath which is accurate within 1° C. An error of 3° could introduce an error of 30 percent with enzyme determinations routinely performed, at least in areas where the curve is linear.

pH. There is a specific pH value at which a particular enzyme is most active. In fact, enzymes are even characterized by the pH at which they are most active, as in the case of acid and alkaline phosphatase. It is a serious error indeed to report enzyme activity without controlling pH, because such an error could cause "normal" results where the activity is actually elevated. The use of carefully prepared reagents or commercial substrates precludes some of the difficulty, although the use of a pH meter and suitable buffers is the best technical approach.

Radiation. Forms of radiant energy, including ordinary sunlight and artificial light, can disrupt the protein molecules to the extent of affecting enzyme activity. Care must be taken in storing enzymes to avoid sources of radiant energy, particularly ultraviolet light.

Inhibitors. Glassware used in enzyme studies must be chemically clean, since the slightest contamination with heavy metals or certain salts will "poison" enzymes. Reagents such as mercuric nitrate, which is used in the Schales chloride procedure, or mercury from the Van Slyke apparatus, must be kept away from enzyme tests. The storage of reagents in metal containers (for example, glycerine for urease suspension) should be avoided.

Time of activity. The period of time during which an enzyme acts will also determine the amount o substrate converted.

Nomenclature. Most of the enzymes first described do not follow any special system of naming. For example, salivary amylase was called ptyalin, from the Greek word for saliva. As enzyme chemistry became more conventional, there developed a system for naming enzymes which is in use today. A complete volume of rules for naming enzymes has been published recently as the result of an international agreement. It is sufficient for our purposes to recognize two of the basic systems commonly used.

a. Enzymes are sometimes named for the substrate upon which they act and are characterized by the ending -ase. Examples are phosphatase and amylase, which act on substrates containing phosphates and starch, respectively (Amylum is Latin for starch.)

b. Enzymes may be named according to their function. An example would be transaminase, which functions in the transfer of an amino group. This appears to be the most meaningful method of naming in use today.

Exercises (225):

1. Why are enzymes called biocatalysts?

2. Since conventional expressions of concentration such as mg/dl would not prove to be a sensitive enough scale for the assay of enzymes, what specifically does the biochemist measure?

3. Define the international unit as a measure of enzyme concentration.

4. What is the advantage of expressing the enzyme activity in international units?

5. Why is the enzyme activity slower in a substrate of low concentration?

6. How does the activity of an enzyme vary with the concentration?

7. What substance does the enzyme act upon to produce a chemical change?

8. What effect does a very high temperature have upon an enzyme?

9. How may an enzyme be stored for a short period of time? Why?

10. How significant is the temperature of a water bath in performing most enzyme procedures?

11. How is the pH of an enzyme substrate maintained?

12. Why would a specimen for transaminase activity deteriorate if placed by a window in direct sunlight for several hours?

13. What effect does contaminated glassware (heavy metals or salts) have on enzymes?

14. Give two ways in which enzymes are named.

226. Identify the amylase and lipase tests for pancreatic function in terms of functions of the enzymes, normal levels, and procedural factors.

Amylase. The enzyme hydrolyzes starch into the smaller units of dextrins and maltose. It is present in saliva and secretions of the pancreas. It is usually assayed in the clinical laboratory from serum or urine. Amylase is sometimes called diastase, and pancreatic amylase is sometimes called amylopsin. It is current practice to refrain from labeling amylase according to the area of the body in which it occurs and simply refer to this enzyme as amylase. This test is most commonly performed as a test for pancreatic function in patients complaining of abdominal pain.

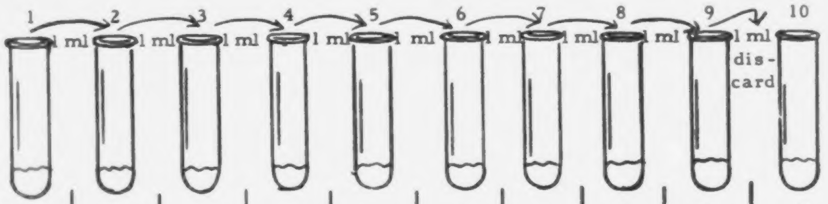
There are various methods of assaying for amylase. The two methods most widely used measure either the amount of end product or the amount of starch remaining after activity. An example of the first technique is the Somogyi method, in which amylase is measured by the amount of reducing substances (calculated as mg per 100 ml plasma or serum) formed from the enzymatic hydrolysis of the starch contained in a starch substrate.

The second is the more popular method in Air Force laboratories and has been modified in many ways. In the Winslow modification, a known quantity of starch is added to serial dilutions of serum, as shown in figure 5-3, and incubated. The amylase present converts starch to sugar. After incubation, iodine solution is added and the tube containing the highest dilution of serum showing complete conversion to sugar (noted by absence of typical starch-iodine blue color) is taken as the endpoint.

In the Carroway modification, the method is based on the measurement of a blue color and serial dilutions are not made. A control tube is run which does not contain amylase and which is used as the basis for calculating the result in Carroway units as follows:

$$\frac{(\text{OD of control} - \text{OD of test})}{\text{OD of control}} \times 800 = \text{Carroway units}$$

One Carroway unit of amylase is defined as the amount of enzyme that will hydrolyze 10 mg of starch in 30 minutes to a stage at which no color is given by iodine. In a micro method, 1.0 ml of reagent containing 0.4 mg of starch is incubated 7.5 minutes with 0.02 ml of



NaCl, 1 Percent (ml)	1	1	1	1	1	1	1	1	1	1
		1 milliliter of:								
Serum	1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Dilution After Serum Transfer	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	
Starch, 0.1 Percent (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Iodine Solution (drops)	3	3	3	3	3	3	3	3	3	3
Titer (units)	4	8	16	32	64	128	256	512	1024	Control

Figure 5-3. Serum amylase dilution procedure (Winslow modification).

serum. This is equivalent to incubating 8000 mg of starch with 100 ml of serum for 30 minutes. If all the starch were hydrolyzed, the serum amylase activity would be 8000/10, or 800 units per 100 ml. The factor of 800 is multiplied by the fraction of starch digested to give the units of amylase activity. Since the equation is valid for any photometer, no calibration is necessary. Normal values by the Carroway method are given as 60 to 160 Carroway units/100 ml of serum.

Procedures involving the starch and iodine reaction usually differ in normal values. As with all enzyme procedures, results should *always* be identified as to the kind of units, such as Winslow units, Carroway units, etc. It is frequently helpful to supply the physician with a list of normal values, or to indicate the normal values on the report form. The term "units" is by no means specific enough. Various hospitals perform enzyme procedures, such as the amylase test, which differ in normal values from a few units to hundreds of units. Few areas of clinical chemistry are in such need of standardization as are enzyme tests, and few enzyme tests enjoy the variety of procedures as the amylase test. In addition to carefully controlling all of the factors which affect enzyme activity, the technician must be very careful to maintain a suitable starch substrate. Improper preparation or contamination with either bacteria or mycotic organisms can render the starch substrate unsatisfactory. Keep in mind, also, that fluorides cannot be used to preserve specimens for enzyme studies.

Lipase. Although many laboratories no longer perform lipase tests, the ability of lipase to split neutral fats to form fatty acids and glycerol may be measured as a test for pancreatic function. In the usual procedure, serum is incubated with an olive oil emulsion substrate. Lipase activity results in splitting of the glyceryl-fatty acid ester bond with the liberation of free fatty acids. The amount of action is determined by titrating the liberated fatty acids with standard alkali, using thymolphthalein as the indicator. A glass electrode pH meter may also be used to detect the endpoint. After a set period of incubation, for 3 hours, the mixture is titrated with 0.05M NaOH to a blue color with thymolpthalein (pH 10.5). The milliliters required to titrate the blank are subtracted from the milliliters required to titrate 1 ml of reacted serum to give the answer in lipase units. A 3-hour incubation should give normal values of 0–167 μM per min (IU) at 37° C. Certain correlations have been shown to exist between lipase and another enzyme, tributyrinase. Some investigators have substituted the assay of tributyrinase for the assay of lipase, but the relationship of the former to pancreatic function is less certain than lipase. Lipase levels of serum are known to show a significant decrease if the serum is hemolyzed, but postprandial or lipid serum has been shown to be quite valid.

Exercises (226):

Match each of the column B items with the statements in column A which most nearly describe it. Each column B item may be used once, more than once, or not at all.

Column A

___ 1. Catalyzes the reaction by which starch is hydrolyzed into maltose and dextrins.
___ 2. Sometimes called diastase.
___ 3. Method in which amylase is measured by the amount of reducing substances formed from the enzymatic hydrolysis of the starch in the starch substrate.
___ 4. A known quantity of starch is added to serial dilutions of serum and incubated.
___ 5. Based on the measurement of a blue color and serial dilutions are not made.
___ 6. The amount of enzyme that will hydrolyze 10 mg of starch in 30 minutes to a stage at which no color is given by iodine.
___ 7. Same as if 8000 mg of starch had been incubated with 100 ml of serum for 30 minutes. Based on incubating 10 mg of starch for 30 minutes 8000/10 gives a factor of 800.
___ 8. Can render the starch substrate unsatisfactory.
___ 9. Cannot be used to preserve specimens for enzyme studies.
___ 10. 60–160 units/100 ml of serum.
___ 11. Serum is incubated with an olive oil substrate.
___ 12. Activity results in splitting of the glyceryl-fatty acid ester bond with the liberation of free fatty acids.
___ 13. After set period of incubation, used to titrate the mixture to a blue color with thymolphthalein (10.5).
___ 14. 0–167 μM per min(IU) at 37° C.
___ 15. Levels of serum may show a significant decrease if the serum is hemolyzed.

Column B

a. Lipase.
b. Amylase.
c. Winslow modification.
d. Somogyi method.
e. Carroway modification.
f. One Somogyi unit.
g. One Carroway unit.
h. Obtain factor of 800 in calculation for the Somogyi micro procedure.
i. Obtain factor of 800 in calculation for the Carroway micro procedure.
j. Slow freezing and slow thawing.
k. Improper preparation and contamination with either bacteria or mycotic organisms.
l. EDTA.
m. Sodium fluoride.
n. Normal values for amylase by Carroway method.
o. 0.05M HCl.
p. 0.05M NaOH.
q. Normal values for lipase.

5-2. Clinically Significant Enzymes

Enzyme tests have proven most useful in the investigation of cardiac, hepatic, pancreatic, muscular, bone, and malignant disorders. Although there are probably hundreds of enzyme tests that can be performed, only the most practical determinations are included in this section.

227. Match each of a list of enzyme procedures with its correct diagnostic use.

General Discussion of Significant Enzymes. As a laboratory technician, you will not be required to make a diagnosis, but you should understand the diagnostic value of the enzyme procedures you perform. The clinician is aided by your results which will enable him to distinguish myocardial infarction from other causes of chest pain. The diagnostic application of serum enzyme assays is based on the accumulated clinical experience of the physician and the conclusions he makes based on accurate information he obtains from your chemistry department. Some enzymes of diagnostic values are listed in table 5-1.

Exercises (227):

Identify the enzymes procedures in column B with the diagnoses to which they are closely related in column A by placing the letter of the column B item beside the number of the column A item. Each column B item may be used once or more than once.

Column A

___ 1. Myocardial infarction and muscle disease.
___ 2. Organo-phosphorus poisoning.
___ 3. Prostatic carcinoma.
___ 4. Myocardial infarction and liver disease.
___ 5. Bone diseases and liver disease.
___ 6. Acute pancreatitis.
___ 7. Obstructive jaundice and hepatic mestastases.
___ 8. Drug induced hemolytic anemia.

Column B

a. Amylase.
b. Lipase.
c. Alkaline phosphatase.
d. Acid phosphatase.
e. LAP.
f. Cholinesterase.
g. SGOT.
h. CPK.
i. Aldolase.
j. 6-GPD.

228. State the purpose for acid and alkaline phosphatase determinations, and identify the methods in terms of pH level, principles, and interpretation of results.

Phosphatases. Increased alkaline phosphatase activity is often associated with liver dysfunction and bone diseases such as rickets. Acid phosphatase is elevated in certain conditions of the prostate. Other pathological conditions, including carcinoma of the breast and Paget's disease, may also be indicated by a rise in acid phosphatase activity. Alkaline phosphatase shows optimum activity at a substrate pH of 9.2 and 9.7, and acid phosphatase is optimum when the pH of the substrate is in the range of 4.9 to 5.1. Actually, measurement of alkaline or acid phosphatase activity involves the measurement of a group of phosphatase enzymes. For example, all of the acid phosphatase of serum is not from the prostate, but may be from the red blood cells or other sources. Further, these components differ somewhat in their chemical makeup.

One of the most widely used methods of determining the activity of serum fractions collectively identified as acid or alkaline phosphatase is by the Shinowara, Jones, and Reinhart method. In this procedure, serum is incubated with buffered glycerophosphate of a definite alkaline or acid pH for 1 hour at 37° C. The optimum pH of 5 for acid phosphatase is easily achieved with a buffer, regardless of variation in the amount of serum used. For alkaline phosphatase an optimum pH of 9.7 is achieved in the final substrate mixture with a buffer of pH 10.8; use of less serum than normally called for in the test requires compensation in the amount of alkaline buffer added. Otherwise, the pH of the final substrate mixture will be significantly higher than 9.7.

The difference between inorganic phosphate before and after incubation is an index of phosphatase activity as measured by the method of Fiske and Subbarow using molybdic and aminonaphthosulfonic acid reagents for color development. When this method is used, it is advisable to report the inorganic phosphorus level in addition to phosphatase activity.

Alkaline phosphatase
Adults: 2.2 to 8.6 mg inorganic phosphorus liberated per hour per 100 ml serum
Children: 3 to 14 mg inorganic phosphorus liberated per hour per 100 ml serum.
Acid Phosphatase
0 to 2.0 mg inorganic phosphorus liberated per hour per 100 ml serum.

Another widely accepted method for phosphatases is outlined in AFM 160–49. The method of Bessey, Lowry, and Brock uses buffered p-nitrophenyl phosphate as a substrate. This compound yields p-nitrophenol upon hydrolysis as follows:

$$\text{p-nitrophenyl phosphate} + \text{H}_2\text{O} \; \text{PO}_{ase} \longrightarrow \text{p-nitrophenol} + \text{H}_3\text{PO}_4$$

The p-nitrophenyl becomes yellow upon the addition of NaOH, whereas p-nitrophenyl phosphate is colorless in either alkaline or acid solution. Intensity of the color is determined to a certain extent by the concentration of NaOH. Many substrates have been studied in connection with phosphatase activity. Some of the substrates are more specific for one type of phosphatase than for another. For example, it has

TABLE 5-1

SERUM-ENZYME DIAGNOSIS

Enzyme	Principal clinical conditions in which enzyme test is of diagnostic value.
Amylase	Acute pancreatitis
Lipase	Acute pancreatitis
Alkaline Phosphatase	Bone disease Liver disease
Acid Phosphatase	Carcinoma of prostate
5'-nucleotidase (5'-N)	Obstructive jaundice Hepatic metastases
Leucine aminopeptidase (LAP)	Obstructive jaundice Hepatic metastases
Cholinesterase	Liver disease Organo-phosphorus poisoning Suxamethonium sensitivity
Glutamate-oxalacetate transaminase (SGOT)	Myocardial infarction Liver disease
Glutamate-pyruvate transaminase (SGPT)	Liver disease
Creatine phosphokinase (CPK)	Myocardial infarction Muscle disease
Lactate dehydrogenase (LDH)	Myocardial infarction
Aldolase	Muscle disease Myocardial infarction
6-Glucose phosphate dehydrogenase (6-GPD)	Drug induced acute hemolytic anemia Evaluate hereditary deficiency resulting in hemolytic anemias.

been shown that alpha-naphthyl phosphate is twice as specific as beta-glycerophosphate for prostatic acid phosphatase.

A third method frequently uses reagents in the form of tablets. While the procedure is not as detailed as the one above, some laboratories use the tablets as a screening test. Alkaline phosphatase levels follow the rate of bone formation, which accounts for the higher level in children. Serum is the specimen of choice, since fluorides and oxalates both act as inhibitors. There is some question of specimen stability, but both alkaline and acid phosphatases are probably stable in the frozen state.

Kinetic methods for alkaline phosphatase have used both p-nitrophenyl phosphate and phenolphthalein phosphate as substrates. A 1- or 2-minute reading with 20 μl of serum is used for the assay.

Methods using phenyl phosphate, β-glycerophosphate, and p-nitrophenyl phosphate as substrates have been adapted to automated system for alkaline phosphatases.

Acid phosphatases determinations have utilized either phenyl or -naphthyl phosphate as a substrate. The systems have incorporated the use of tartrate or copper ions to differentiate the prostatic and erythrocytic acid phosphatases.

5′-Nucleotidase (5′NT). This enzyme is an alkaline phosphomonoterase which specifically hydrolyzes nucleotides with a phosphate radical attached to the 5′ position of the pentose, for example, adenosine monophosphate. Increased values are noted in malignant or granulomatous disease involving liver, and in hepatic cirrhosis. Compared with alkaline phosphatase, 5′-nucleotidase shows a similar trend of increased values in both obstructive and hepatic jaundice.

Exercises (228):

1. Increased alkaline phosphatase activity may be associated with what diseases?

2. What is the most common clinical reason for requesting acid phosphatase tests?

3. Name the optimal pH of the substrate for determining
   a. acid phosphatase.
   b. alkaline phosphatase.

4. A test for acid phosphatase is ordered on a female patient. What dysfunction may be indicated?

5. What is the principle of the alkaline and acid phosphatase procedures by the Shinowara, Jones, and Reinhart?

6. What is the principle of the alkaline and acid phosphatase procedures by Bessey, Lowry and Brock?

7. A specimen is submitted in lithium oxalate for acid phosphatase. The patient cannot be recalled and you are asked if the plasma is suitable. What would you reply?

8. What reagents are used as substrates in the automated methods for alkaline phosphatase?

9. If a 5′-nucleotidase (5′ NT) was requested, what possible conditions would be suspected when values are increased?

229. Cite diagnostic value, levels, methods, interpretation, and shipping procedures for the determination of transaminases (SGOT, SGPT), lactic dehydrogenase (LDH), and creatine phosphokinase (CPK).

Transaminases. The two principal transaminase enzymes for which blood is analyzed in the clinical laboratory are serum glutamicoxalacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT).

SGOT. This test is of greatest diagnostic value in cases of myocardial infarction. It is the moe commonly performed of the two transaminase procedures discussed here. In theory, SGOT catalyzes the following biochemical reaction which takes place in tissues, including cardiac tissue:

$$\text{alpha-ketoglutarate} + \text{L-asparate} \xrightarrow{\text{SGOT}} \text{glutamate} + \text{oxalacetate}$$

Many, if not most, laboratories use a commercially prepared substrate and color developer for this procedure because reagents that have not been stabilized are more sensitive to temperature fluctuations. This can be a source of significant error.

The various procedures available are not identical, some being more involved than others. In the method of Babson *et al*, outlined in AFM 160-49, the oxalacetate is coupled with a stabilized dizonium salt to form a red dye which is proportional to the SGOT activity. The normal values are 9-32 μM per liter per min (IU) at 37° C. If the enzyme activity exceeds 350 units, the test should be repeated using 1:5 dilution with saline. The procedure is available in kit form as a standard item. The SGOT level is always increased in acute myocardial infarctions and is also elevated in cases of hepatocellular damage.

SGPT. Primarily a test for liver function, the procedure for glutamic-pyruvic transaminase measures the biocatalyst in the following reaction:

$$\text{alpha-ketoglutarate} + \text{L-alanine} \xrightarrow{\text{SGPT}} \text{glutamate} + \text{pyruvate}$$

The procedure and reagents are frequently identical with the SGOT procedure, except that a different substrate is used. Color development mixtures are interchangeable if the procedures are the same except for substrate. Normal values for serum SGPT levels given in AFM 160-49 are 5-30 μM per liter per min (IU) at 37° C.

Cerebrospinal fluid values are normally lower for both SGOT and SGPT. It is also reported that diagnostic importance can be attached to the ratio of serum SGOT to SGPT, the point being that SGPT values are higher than SGOT values only in cases of viral hepatitis. Salicylates produce an increased GPT in children but not in adults. Determination of GOT, GPT, or both is useful in the early recognition of viral or toxic hepatitis and is, therefore, helpful in studying patients exposed to hepatotoxic drugs. Elevation of the SGPT level appear to reflect acute hepatic disease somewhat more specifically than do GOT values. Values of GPT are modestly elevated in most patients with posthepatic jaundice, intrahepatic cholestasis, metastatic carcinoma, cirrhosis, or alcoholic steatonecrosis (alcoholic hepatitis). Levels of GPT are normal or only minimally elevated in patients with myocardial infarction.

Although interpretation is the responsiblity of the medical profession, it is a matter of concern for the technician to submit data which differentiates minimal but significant changes in the serum transaminase levels. Serum showing high transaminase values should be diluted and rerun. All of the factors outlined in the first part of this chapter must be considered as potential sources of error. Serum samples for SGPT studies are stable in the refrigerator for 2 to 3 days if the cells are separated, and samples for SGOT are stable somewhat longer. Storage time may be lengthened if the serum is frozen. In both tests, slight hemolysis will not elevate results beyond confidence limits, but very obvious hemolysis will produce elevated results.

Lactic Dehydrogenase (LDH or LD). This enzyme catalyzes the conversion of pyruvic acid to lactic acid, as well as the reverse reaction shown as follows:

$$\text{Pyruvic acid} + \text{beta-DPNH} \overset{\text{LDH}}{\rightleftharpoons} \text{beta-DPN} + \text{lactic acid}$$

The term "DPN" stands for diphosphopyridine nucleotide, and "DPNH" for the reduced form of the nucleotide. The rate at which the concentration of DPNH decreases under conditions of the test is measured directly as a change in optical density with a spectrophotometer. This test has gained wide acceptance in the past few years and is now supplied in the form of prepared reagents through medical supply channels.

Lactic dehydrogenase is remarkably stable in serum separated from cells even at room temperature. However, refrigeration is recommended for an extended period of storage up to a week. Specimens to be stored for more than a week should be frozen. Hemolysis has a profound effect on this test, since the activity of the enzyme in red cells is some 100 times that of serum, so hemolyzed serum must never be used. It has been reported that heparin and EDTA do not interfere with the test but that oxilate inhibits LDH. Investigators have discovered that the LDH activity in both heparinized and oxalate samples were 50 percent higher than the activity in serum; therefore, the analysis should be restricted to serum. The normal serum level is less than 500 units per ml (240 International Units per liter) at 25° C, using the method of Wroblewski and La Due, which measures the reduction of pyruvate to lactate. The reagent kits for LDH are available from Sigma Chemical Company and Dade Reagents. The normal values are 214 to 535 μM per liter per min (IU) at 37° C.

Following myocardial infarction, the incidence of LDH elevation is even greater than that of SGOT and CPK. A major advantage of LDH determination is that its elevation is much more prolonged and is thus of special value if initial blood samples cannot be obtained until some days after the infarctive episode. The LDH level of spinal fluid is elevated in certain conditions of the central nervous system. LDH isoenzymes can be determined by starch gel electrophoresis. This procedure should be sent to a reference laboratory. The serum should be shipped at a temperature range of 0°-4° C; it should never be shipped frozen. The determination should be made within 24 hours.

Creative Phosphokinase (CPK). Reported to be of value in the diagnosis of muscular dystrophy, myocardial infarction and hypothyroidism, CPK catalyses the following reaction:

$$\text{ATP} + \text{creatine} \underset{}{\overset{\text{CPK}}{\rightleftharpoons}} \text{ADP} + \text{phosphocreatine}$$

Serum CPK activity is raised in all incidences of muscular dystrophy. Determination of the serum CPK has proved more sensitive than any other enzyme procedure in the investigation of skeletal muscle disease, and is also valuable in the diagnosis of myocardial infarction. Blood should not be collected for CPK determination within 48 hours of severe or prolonged exercise since this can result in elevated levels. Over 90 percent of patients with myocardial infarction show a rise in CPK values. Some sources of error include the following:

a. Constant temperature (37° C) must be maintained, or variable results will be obtained.

b. Exact timing is critical in order to obtain accurate results.

c. Serum with appreciable hemolysis should not be used.

d. Anticoagulants can inhibit CPK activity; therefore, serum must be used.

e. Any intramuscular injection will transiently elevate the CPK level due to damage of the muscle.

If the laboratory cannot perform the test, the sample should be stored at 0°-4° C and shipped frozen to the area reference laboratory. Normal values are:

a. Male: 5-50 units per liter at 30° C.

b. Female: 5-30 units per liter at 30° C.

Exercises (229):

1. What is the primary diagnostic value of: (a) SGOT; (b) SGPT?

2. It is brought to your attention that SGOT values in your laboratory are nearly always higher than SGPT values when both tests are performed on the same patient. How would you evaluate this situation?

3. At what elevated level should the SGOT be repeated and what dilution is suggested?

4. How would hemolysis affect the results of the SGOT and SGPT?

5. How stable is the LDH in serum that has been separated from the cells?

6. What effect does oxalate have on LDH?

7. What major advantage does the LDH determination serve?

8. How should the serum for LDH isoenzymes be shipped?

9. How long does the reference laboratory have to complete the determination of the LDH isoenzymes?

10. What is the primary diagnostic value of CPK?

11. What effect does intramuscular injection have on the CPK results and why?

12. What effect does prolonged exercise have on the CPK for at least 24 hours?

13. If your laboratory cannot perform a CPK, how should you store and ship the specimen to the reference laboratory?

14. How will anticoagulants affect the CPK results?

230. Indicate whether given statements correctly reflect the purposes for the leucine aminopeptidase (LAP) and Glucose-6-Phosphate Dehydrogenase (G-6-PD) tests, specimen preservation, storage, principles of tests, and methods of analysis.

Leucine Aminopeptidase (LAP). Serum LAP is assayed to rule out or diagnose carcinoma of the pancreas and certain other conditions, including acute pancreatitis, malignancies, and liver disease. It is also elevated in the third trimester of pregnancy, and following some surgical procedures. Diagnosis frequently depends upon a comparison of urine and serum LDH values and upon the changes in serum

LDH as the condition progresses. Nonmalignant causes tend to produce a more transient rise in serum LDH values. The enzyme LAP hydrolyzes certain leucine compounds and their derivatives. A common laboratory procedure uses a leucylnaphthylamide substrate proposed by Goldbarg and Rutenburg. The reaction is as follows:

$$\text{L-leucyl-beta-naphthylamide} + H_2O \xrightarrow{\text{LAP}} \text{leucine} + \text{beta-naphthylamine}$$

The beta-naphthylamine which is formed is then assayed by the diazo reaction. LAP enzyme is reported to be stable in serum or urine up to 7 days at 4° C and for several weeks if frozen.

Significantly increased serum leucine aminopeptidase (LAP) activity is found in obstructive jaundice and carcinoma of the pancreas. Moderate elevation occurs in acute pancreatitis, acute hepatitis, hepatic cirrhosis, cholecystitis, and in the presence of carcinomatous metastases in the liver. The enzyme is a more sensitive indicator of hepatic metastases than alkaline phosphatase in both jaundiced and nonicteric patients. LAP is not elevated in bone disease or metastases in bone. The upper limit of normal in serum is 230 Goldbarg-Peneda-Rutenburg units in males (55 International Units/liter at 37° C) and 210 units in females (50 International Units/liter at 37° C).

Urine normal values are as follows:

	24-Hour Urine
Male	50 to 175 G-R Units
Female	20 to 70 G-R Units

Glucose-6-Phosphate Dehydrogenase (G-6-PD). G-6-PD is the erythrocyte enzyme most commonly determined for the study of enzyme deficiency. It catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconolactone, which immediately hydrolyzes to 6-phosphogluconate.

$$\text{Glucose-6-phosphate} + \text{NADP} \longrightarrow \text{6-Phosphogluconate} + \text{NADPH} + H^+$$

Another enzyme present in the red cell catalyzes the further oxidation of 6-phosphogluconate to 5-ribulose phosphate and CO_2.

$$\text{6-Phosphogluconate} + \text{NADP}^+ \longrightarrow \text{Ribulose-5-phosphate} + CO_2 + \text{NADPH} + H^+$$

In the assays for G-6-PD, the two reactions are not distinguished and the overall reaction is measured.

This enzyme is presented as an example of an enzyme which has gained the attention of clinical laboratories in the past few years. Acute hemolytic anemias can be induced by the use of certain drugs in persons having a deficiency or decreased activity of glucose-6-phosphate dehydrogenase in their red blood cells. Many drugs are known to induce hemolysis, including primaquine drugs used in the treatment of malaria, various nitrofurans, some sulfonamides, acetophenetidin and others.

Individuals who develop hemolytic anemias frequently present a diagnostic problem as to the etiology of the anemia. It is extremely helpful to the physician if you are able to demonstrate an enzyme deficiency which can draw attention to the effect of drugs as a causative factor.

Approximately 10 percent of male American blacks who were given 30 mg of primaquine a day developed a self-limited, acute hymolytic anemia. The older red cells were destroyed and the younger cells unaffected, and it was found that the deficiency in the susceptible red cells was G-6-PD.

It has been since discovered that G-6-PD deficiency is widespread throughout the world. Among Caucasians, the highest incidence is in Mediterranean people; the deficiency is also found in black and in Orientals.

Since G-6-PD is determined by a gene on the X-chromosome, full expression of the deficiency is found in the male hemozygote. Partial expression may be found in the heterozygote female who has two populations of red cells, one normal and one deficient. The deficiency of G-6-PD results in a limitation of the regeneration of NADPH, which renders the cells vulnerable to oxidative denaturation of hemoglobin. Glucose-6-phosphate dehydrogenase is normally highest in young cells and decrease as the cell ages, in persons with the deficiency, the older cells are preferentially destroyed. Blacks have a milder deficiency than Caucasians, have fewer cell types of the body affected and as a rule, do not have chronic hemolytic anemias. The rarer forms of G-6-PD hemolytic anemias are more commonly found in people of northern European extraction. Caucasian with G-6-PD deficiency, exposed to fava beans, results in a severe and sometimes fatal hemolytic reaction (favism) in which serum factors are also involved.

Screening tests for G-6-PD deficiency. The several types of tests used in screening for G-6-PD deficiency vary in their specificity, sensitivity to the heterozygous state, and the amount and freshness of blood required.

Heinz body tests for G-6-PD deficient cells. Acetylphenylhydrazine, an oxidant drug, is added to the patient's and control blood samples. After 2- and 4-hour incubation at 37° C, the samples are examined for Heinz bodies. Control values should be 0 to 30 percent. Values for persons with G-6-PD deficiency (or defects in the gluthathione system or unstable hemoglobin) will be greater, usually over 45 percent.

Dye reduction test of Motulsky. The test is usually performed using commercially available kits. A mixture of glucose-6-phosphate, NADP, and brilliant cresyl blue dye in this buffer is incubated with hemolysate. If G-6-PD is present, the NADP will be reduced to NADPH which, in turn, will reduce the blue dye to its colorless form. The reduction time is noted for the patient's blood and for that of a normal control with an identical hemoglobin concentration. The noted time is inversely proportional to the amount of G-6-PD present and is prolonged in G-6-PD deficient subjects.

This test is quite specific and can be performed on stored blood. It has the advantage that it can be performed on microsamples, but is likely to yield false negative results in heterozygotes and black males with G-6-PD deficiency during a hemolytic episode.

Fluorescence of NADPH. Whole blood is added to a mixture of glucose-6-phosphate, NADP, saponin, and buffer, and a spot of this mixture is placed on filter paper and observed for fluorescence with ultraviolet light.

Quantitative determination of G-6-PD. Most quantitative determinations are based on the rate of reduction of NADP to NADPH and measured spectrophotometrically at 340 nm when a hemolysate is incubated with G-6-PD. Since NADPH is formed in the first two reactions of the hexose monophosphate shunt, and assay for 6-GPD activity is often done simultaneously.

G-6-PD is stable in erythrocytes preserved with Alsever's solution for 3.5 weeks at 30° C. While blood collected in glucose-EDTA (1 mg EDTA and 5 mg glucose per ml of blood) is stable for 8 days at room temperature and 30° C. Hemolysates used are not stable for more than a few hours at room temperature or 4° C.

Exercises (230):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

T F 1. Serum LAP is assayed to rule out or diagnose carcinoma of the pancreas, acute pancreatitis, malignancies, and liver disease.

T F 2. The enzyme LAP hydrolyzes certain cystine compounds and their derivatives.

T F 3. LAP enzyme is reported to be stable in serum or urine up to 7 days at 4° C and for several weeks if frozen.

T F 4. LAP is also elevated in bone disease or metastasis in bone.

T F 5. The enzyme LAP is a more sensitive indicator of hepatic metastases than alkaline phosphatase.

T F 6. G-6-PD is the erythrocyte enzyme most commonly determined for the study of enzyme deficiency.

T F 7. The G-6-PD enzyme catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconolactone which immediately hydrolyzes to ribulose-5-phosphate.

T F 8. Acute hemolytic anemias can be induced by the use of certain drugs in persons having a deficiency or decreased activity of glucose-6-phosphate dehydrogenase in their red blood cells.

T F 9. The deficiency of G-6-PD results in a limitation of the regeneration of DPNH, which renders the cells vulnerable to oxidative denaturation of hemoglobin.

T F 10. In the Heinz body tests for G-6-PD deficient cells, values for persons with G-6-PD deficiency will be greater than 25 percent.

T F 11. The dye reduction test of Motulsky is not specific and requires only the use of fresh blood.

T F 12. Most quantitative determinations of G-6-PD are based on the rate of reduction of DPN DPNH and measured at a wavelength of 340 nm.

T F 13. G-6-PD is stable in erythrocytes preserved with Alsever's solution for 3.5 weeks at 30° C.

T F 14. Hemolysates used are quite stable for 8 days at room temperature and 30° C.

231. Identify methods used for trypsin-duodenal assays and trypsin-feces assays.

Trypsin. Trypsin levels are most commonly assayed from fecal or duodenal contents because only limited success has been reported in correlating the trypsin level of serum with clinical disorders. One situation in which a trypsin level may be helpful results when there is a decrease in duodenal enzymes due to obstruction. On the other hand, some of the other enzymes, particularly lipase, cannot be accurately assayed from fecal or duodenal contents.

Various methods are used for trypsin analysis. In the method of Gross, casein (which is precipitated by acetic acid) is digested by trypsin, forming products which are not precipitated by acetic acid. The result is reported in terms of trypsin activity equivalent to the reciprocal of the number of milliliters of duodenal fluid in the first clear tube. A popular technique widely used in Air Force laboratories is to prepare a series of dilutions of a fecal specimen and then measure the capacity of trypsin to dissolve the gelatin of X-ray film.

A method of screening trypsin duodenal contents is outlined in AFM 160-49. To each of a series of ten tubes, add 10 ml of casein solution, which has been heated to 40° C. Add increasing amounts of clear duodenal fluid to these tubes, varying from 0.1 to 1.0 ml, at intervals of 0.1 ml. Incubate the tubes at 37° C for 15 minutes, remove them from the incubator, and acidify them by adding a few drops of 1 percent acetic acid to each tube. The tubes in which casein is completely digested will remain clear when acidified. Tubes with incomplete digestion will show some turbidity. The tryptic activity is calculated by selecting the first tube in the series which exhibits no turbidity upon the addition of acid.

Tryptic activity $= \frac{1}{x}$ (x equals the number of ml of duondenal fluid in the first tube to appear clear). For example, the first tube showing no turbidity contains 0.6 of duodenal fluid. The tryptic activity will be $\frac{1}{0.6} =$ 1.6. The average tryptic activity by this method is 2.5.

Another method of trypsin assay outlined in AFM 160-49 is trypsin in feces. The trypsin in feces digests the gelatin on X-ray film, leaving the bare celluloid backing. Two ml of 5 percent sodium bicarbonate is placed into each of ten tubes. Into tube 1, place a 1:4 dilution of fecal suspension which is 3 ml of 5 percent sodium bicarbonate and 1 gm of feces. Thoroughly mix the 1:4 fecal suspension with 2 ml of sodium bicarbonate. Transfer 2 ml of fecal suspension from tube 1 to tube 2 and mix. Repeat for subsequent tubes in the series. Insert a strip of plain, unexposed, and unfixed dental X-ray film in each tube. Place a strip of film into a tube containing 2 ml of sodium bicarbonate solution, which serves as a control tube. Incubate all tubes for 1 hour at 37° C. Wash the strips gently in cold running water. The gelatin on the film will be digested if tryptic activity is present. The tube with the highest dilution is recorded in which digestion occurs.

Some sources of errors include (a) contamination with urine, which may yield a false positive; (b) stools over a day old, which may give false positive due to bacterial action; and (c) failure to start the test within a reasonable time after collection.

Normally, children are expected to show some degree of tryptic activity in the feces, the amount decreasing with age. Adults have less tryptic activity in the feces than do infants.

Exercises (231):

Match the methods in column B with the procedures, reagents, and sources of errors in column A. Each item in column B may be used more than once.

Column A	*Column B*
___ 1. 5 percent sodium bicarbonate.	a. Trypsin-duodenal content.
___ 2. Insert strip of plain, unexposed, and unfixed dental X-ray film.	b. Trypsin-feces.
___ 3. Series of tubes are incubated for 15 minutes.	
___ 4. Specimen mixture of casein solution is acidified with a few drops 1 percent acetic acid.	
___ 5. Contamination with urine may yield a false positive.	
___ 6. Tryptic activity $= \frac{1}{x}$.	

232. State definitions, terms, uses, and methods in isoenzyme determinations.

Isoenzymes. There are many enzymes in a variety of molecular forms, which differ in their physical and chemical properties, yet show similar substrate specificity. They are known as the isoenzyme forms of the enzyme. A number of physical and chemical procedures are available for isoenzyme separation and recognition, but for clinical purposes isoenzymes are generally separated by electrophoresis. An enzyme may be present as different isoenzymes in different tissues, or the enzyme of a single tissue may be separable into a number of isoenzyme fractions. Many of the serum enzymes used in the investigation of disease may originate from one of several tissues. Isoenzyme determination permits more precise recognition of their tissue of origin than is possible solely by the determination of total serum enzyme activity. Human lactic dehydrogenase (LDH) can serve as an example. It can be separated by electrophoresis into five fractions, and these isoenzymes all catalyse the same reaction: the conversion of pyruvate to lactate. Different organs contain characteristic LDH isoenzymes.

Only the isoenzymes of the lactic dehydrogenase and of the alkaline phosphatase of the serum have been found to have important clinical relevance.

Lactic Acid Isoenzymes. Each of these isoenzymes can be distinguished from the others by serologic, electrophoretic, and various other chemical tests. The observations on the multiple molecular forms of lactic dehydrogenase have indeed been of great current interest in isoenzymology. The isoenzymes of LD are designated in ordinary usage according to their electrophoretic mobility. The fraction with the greatest mobility (anodic) is called LD$_1$, the one with the least anodic mobility is called LD$_5$, the others are designated LD$_2$, LD$_3$, LD$_4$, respectively. Isoenzymes from elevated serum LD levels of various diseases have revealed abnormal patterns that reflect the tissues involved. Below is a list of some enzymes for which isoenzymes have been demonstrated.

Malic dehydrogenase (MD).
Lactic dehydrogenase (LD).
Isocitric dehydrogenase (ICD).
G-6-PD.
Glutamate-oxalacetate transaminase (GOT).
Creatine phosphokinase (CPK).
Acetylcholinesterase.
Cholinesterase.
Alkaline phosphatase.
Acid phosphatase.
5-nucleotidase (5'-N).
Leucine aminopeptidase (LAP).
Amylase.

Exercises (232):

1. What are isoenzymes?

2. What method of analysis is used to determine isoenzymes?

3. How does isoenzyme determination enable more precise diagnosis of disease?

4. How are the isoenzymes of LD designated?

233. Identify the principle of other given enzymes, use for diagnosis, conditions for collection, storage and shipment.

Other Enzyme Procedures. Other enzyme procedures that are currently available in kit form include the following:

a. Malic Dehydrogenase (MDH). It is believed that MDH levels follow a pattern similar to SGOT levels.

b. Sorbitol Dehydrogenase (SDH). This enzyme catalyzes the following reaction:

$$\text{D-fructose} + \text{beta-DPNH} \xrightarrow{\text{SDH}} \text{D-sorbitol} + \text{beta-DPN}$$

Elevated levels of SDH have been reported in liver disease, myocardial infarction, and diabetes.

c. Alpha-Hydroxybutyric Dehydrogenase. This enzyme functions in the conversion of alpha-ketobutyric acid to alpha-hydroxybutyric acid. Elevated levels of this enzyme are usually found in conditions which result in elevated LDH levels. This procedure is also helpful in cases of muscular dystrophy and diseases of the pharynx (angina).

d. Phosphohexose Isomerase. The enzyme phosphohexose isomerase catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate. Although not yet firmly established as a clinical procedure, the assay of this enzyme may aid in the diagnosis of carcinoma and viral hepatitis.

e. Ornithine Carbamyl Transferase (OCT). Since this enzyme occurs almost exclusively in liver cells, elevation of OCT may be of diagnostic value in estimating liver cell damage. It catalyzes the conversion of citrulline and phosphate to ornithine and carbamyl phosphate.

f. Cholinesterase. The enzyme cholinesterase hydrolyzes various choline esters. It is of more or less diagnostic value in poisoning from organic phosphates which are present in certain insecticides and which cause a reduction in the serum cholinesterase activity. Results on serum are identical to those obtained on plasma. Citrate, oxalate, and fluoride must be avoided because of complexation of divalent ions, such as Ca^{2+}, which activate cholinesterase. Heparin is satisfactory as an anticoagulant. The serum should be separated from the clot within 2 hours. Specimen may be shipped at 4° C.

g. Cytochrome Oxidase. This enzyme is sometimes assayed from tissues as a histochemical procedure.

h. Isocitric Dehydrogenase (ICD). Reported to be of value in the diagnosis of liver cell damage, this enzyme catalyzes the following reaction:

$$\text{d-Isocitrate} + \text{TPN} \xrightarrow[\text{ICD}]{Mn^{++}} \text{gamma-ketoglutarate} + CO_2 + \text{TPNH}$$

An intermediate product may be formed before CO_2 and TPNH. The ICD level may be elevated in a variety of diseases.

i. Aldolase. There is evidence that assay of aldolase levels may be of value in the diagnosis of muscular dystrophy, myocardial infarctions, and carcinoma of the prostate. Aldolase catalyzes the conversion of fructose-1, 6-diphosphate to dihydroxyacetone phosphate plus glyceraldehyde-3-phosphate. Plasma is a more satisfactory specimen because platelets contain large amounts of the enzyme. Serum can be used, if necessary. Specimens must be shipped frozen as they are stable for 4 days in a frozen state.

It is to be expected that many other enzymes will be investigated in the near future, and that more specific correlations will be drawn between their activities and metabolic or other clinical disorders.

Exercises (233):

Match each of the column B items with the statements in column A which most adequately describe it. Each column B item may be used once, more than once, or not at all.

Column A

___ 1. Elevated levels reported in liver disease, myocardial infarction, and diabetes.
___ 2. Levels follow a pattern similar to SGOT levels.
___ 3. Catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate.
___ 4. Helpful in cases of muscular dystrophy and diseases of the pharynx (angina).
___ 5. Elevation may be of diagnostic value in estimating liver cell damage.
___ 6. Diagnostic value in poisoning from organic phosphates which are present in certain insecticides.
___ 7. Catalyzes the conversion of citrulline and phosphate to ornithine and carbamyl.
___ 8. The serum should be separated from the clot within 2 hours and the specimen may be shipped at 4° C.
___ 9. Assayed from tissues as a histochemical procedure.
___ 10. May be of value in diagnosis of muscular dystrophy, myocardial infarctions, and carcinoma of the prostate.
___ 11. Plasma is a more satisfactory specimen because platelets contain large amounts of the enzyme.
___ 12. Specimens must be shipped frozen as they are stable for 4 days in a frozen state.

Column B

a. Malic dehydrogenase.
b. Sorbitol dehydrogenase.
c. Cholinesterase.
d. Ornithine carbamyl transferase.
e. Cytochrome oxidase.
f. Aldahase.
g. Isocitric dehydrogenase.
h. Alpha-hydroxybutyric dehydrogenase.
i. Phosphohexose isomerase.

234. Indicate the basic principle for ultraviolet enzyme determination, range of wavelength, reaction temperature, and comparison with colorimetric methods.

Ultraviolet Method of Enzyme Determinations. Enzyme assays which require kinetic measurements in the ultraviolet wavelength region can introduce minute problems for many clinical laboratories if proper equipment is not used. Most of these procedures depend on the absorption at 340 mμ (nm) of pyridene nucleotides ($NAD \rightarrow NADH_2$). Since there is an increasing number of nucleotide-dependent enzymes of clinical importance, the ability to work in the 340 nm range is becoming of great importance. It is, therefore, essential that you obtain a spectrophotometer which measures accurately in the ultraviolet range. A number of inexpensive spectrophotometers with a preset wavelength in the UV range are now available with stable built-in thermal control and appear to be quite suitable for routine enzyme assays.

For example, the lactic dehydrogenase procedure emphasizes the salient feature of clinical enzyme assays. In the pH range 7 to 8, the equilibrium favors reduction of pyruvate to lactate, whereas the reverse reaction is favored in the pH range 9 to 10. Authorities have reported a lactic acid dehydrogenase assay incorporating a buffer at pH 8.8, lactate as the substrate, and NAD as the coenzymes. The enzyme is provided in the serum, and the assay is conducted at 25° C. Spectrophotometric measurements of absorbance are made each minute for 5 minutes at wavelength 340 mμ. Lactate is oxidized to pyruvate with conversion of coenzyme (NAD) to reduced enzyme ($NADH_2$). The assessment of adherence to zero-order reaction is accomplished by multiple measurements at 1-minute intervals.

Many commercial sources have ultraviolet reagent systems that are prepared as convenient, integrated systems for in vitro enzyme analysis. Usually, all necessary reagents are contained in one vial and require only the addition of distilled water or buffered substrate to prepare them for use. The materials are freeze-dried and stoppered under vacuum to insure maximum stability under refrigeration prior to use.

The reaction temperature for all enzyme reactions is a parameter that must be carefully controlled to obtain accurate results. The temperatures recommended for these procedures range from 25° C to 37° C. The reaction must be linear; that is, the absorbance change per minute must be constant within time to obtain accurate results.

Ultraviolet vs. Colorimetric Methods. Technically, the diagnostic enzyme products can be divided into two groups: those that measure enzyme activity and those that measure other biological fluid constituents via enzyme assays. For the measurement of enzyme activity, the ultraviolet kinetic method is considered the most advanced. For the determination of other constituents, the ultraviolet endpoint determination currently provides the best reliability. Colorimetric methods falter in that they lack specificity and are unreliable in this respect.

For example, the colorimetric determination of SGOT in which the produced oxalacetate is measured, is either nonspecific or inaccurate, or both. This is so, not only because other keto acids interfere with the determination, but also because the reaction product oxalacetate inhibits the enzymatic transamination. In contrast, the colorimetric methods using the NADH coupled-removal of oxalacetate not only match, but may even surpass, the accuracy of the commonly used ultraviolet methods. With the ever-increasing demand by laboratory professionals and the FDA for greater reliability insure the best possible patient care, many significant changes have taken place in the reagent industry.

With the advent of phenazine and diaphorase catalyzed reduction of tetrazolium salts by NADH, the laboratory may obtain colorimetric results comparable to those obtained in the ultraviolet modes.

Despite great progress made in clinical enzymology, there is still much to be investigated in the resolution of enzyme components.

Exercises (234):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

T F 1. Ultraviolet enzyme procedures depend on the absorption at 340 nm of diphosphopyridine nucleotide (DPN).

T F 2. A spectrophotometer which measures in the ultraviolet range is necessary for accurate determination of UV enzymes.

T F 3. The assessment of adherence to zero-order reaction is accomplished by multiple measurements at 5-minute intervals.

T F 4. The temperature range recommended for these UV enzyme determinations is from 25° C to 47° C.

T F 5. For measurement of enzyme activity, the ultraviolet kinetic method is considered the least advanced.

T F 6. For enzyme determination of the constituents, the colorimetric methods lack specificity and are unreliable.

T F 7. In the SGOT determination in which the produced oxalacetate is measured, the reaction is nonspecific or inaccurate because the reaction product oxalacetate inhibits enzymatic transamination.

T F 8. Colorimetric methods using NADH coupled-removal of oxalacetate may surpass the accuracy of the commonly used ultraviolet methods.

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NOTE: None of the items listed in the bibliography above are available through ECI. If you cannot borrow them from local sources, such as your base library or local library, you may request one item at a time on a loan basis from the AU Library, Maxwell AFB AL 36112, ATTN: ECI Bibliographic Assistant. However, the AU Library generally lends only *books* and a limited number of *AFMs*. TOs, classified publications, and other types of publications are *not* available.

ANSWERS FOR EXERCISES

CHAPTER I

Reference:

200 - 1. b.
200 - 2. f.
200 - 3. i.
200 - 4. c.
200 - 5. c.
200 - 6. e.
200 - 7. j.
200 - 8. j.
200 - 9. g.
200 - 10. d.

201 - 1. Most of the sodium is found in fluids, particularly plasma, but large amounts are also found in the skeletal system.
201 - 2. A loss of fluid in the body can hide a loss of sodium. Water retention can hide an increase in total body sodium.
201 - 3. Osmotic equilibrium, cell permeability, and muscle irritability.
201 - 4. Most potassium is within the body cells, but some is present in the extracellular fluid.
201 - 5. Result would be higher because of potassium release from ruptured cells.
201 - 6. Disease of the heart and central nervous system.
201 - 7. Diarrhea; cell damage, particularly cells of the kidney tubules.
201 - 8. There must necessarily be more sodium ions and fewer chloride ions.
201 - 9. Malabsorption of gastric juice or diminished production.
201 - 10. There will be an elevation of the serum bicarbonate to maintain electrolyte balance.
201 - 11. There is a decrease in serum bicarbonate which results in acidosis.

202 - 1. Nitric acid lowers pH and prevents formation of a color complex.
202 - 2. For reasons of consistency with other electrolyte values.
202 - 3. The excess silver ions are titrated with a thiocyanate.
202 - 4. The chloride in solution is titrated with silver ions, which are formed at a constant rate by passage of an electric current.
202 - 5. For early diagnosis and treatment of children with cystic fibrosis.
202 - 6. To stimulate sweat gland secretion.

203 - 1. To enable the physician to evaluate buffering capacity of the blood to maintain a pH of 7.4.
203 - 2. 20:1.

203 - 3. The ionized bicarbonate (HCO_3^-) and the carbonate (CO_3^{--}).
203 - 4. Carbonic acid.
203 - 5. An ionized fraction that contains bicarbonate (HCO_3) and carbonic compounds (CO_3^{--}) and an un-ionized fraction that contains carbonic acid ($HHCO_3$) and physically dissolved (anhydrous) CO_3.
203 - 6. It serves as an index of the amount of CO_2 that can be bound by serum, plasma, and whole blood as HCO_3^- at a pCO_2 of 40 mm at 25° C.
203 - 7. The pressure of a mixed gas, the sum of the partial pressure of the individual gases. That part of the pressure which is contributed by CO_2—partial pressure of CO_2 (pCO_2).
203 - 8. The symbol for hydrogen, H, with the positive ionic sign, both inclosed in brackets, means hydrogen ion concentration.
203 - 9. The symbol for hydrogen, H, without the positive sign means the neutral element hydrogen. However, with the charge symbol, H^+, it means hydrogen ions.

203 - 10. The ratio of the product of hydrogen and bicarbonate ion concentrations to the concentrations of carbonic acid.

204 - 1. F. 6.1.
204 - 2. T.
204 - 3. T.
204 - 4. F. Lower partial pressure.
204 - 5. F. Heparin.
204 - 6. T.
204 - 7. T.
204 - 8. F. Two. One high and one low.
204 - 9. T.

205 - 1. b.
205 - 2. d.
205 - 3. a.
205 - 4. c.
205 - 5. f.
205 - 6. e.
205 - 7. i.
205 - 8. h.
205 - 9. g.
205 - 10. j.
205 - 11. h.

206 - 1. The diagnosis of acidosis or alkalosis cannot be determined with certainty from carbon dioxide levels alone.
206 - 2. The blood pH must be measured within narrow limits.
206 - 3. The hydrogen electrode.
206 - 4. It is important to know the nature of the electrode because each has characteristic capabilities and limitations and should be used to measure the pH of solutions for which it was designed.
206 - 5. Electromotive forces.
206 - 6. Carbon dioxide would be removed by the hydrogen from which the standard electrode is prepared.
206 - 7. Mercury.
206 - 8. Glass electrode.
206 - 9. Temperature.
206 - 10. Contamination, especially when accessible to several people.

207 - 1. b.
207 - 2. c.
207 - 3. b.
207 - 4. d.
207 - 5. a.
207 - 6. a, b.
207 - 7. e.
207 - 8. f.

207 - 9. g.
207 - 10. a.

208 - 1. b.
208 - 2. c.
208 - 3. f.
208 - 4. f.
208 - 5. f.
208 - 6. g.
208 - 7. i.
208 - 8. h.
208 - 9. e, f, d.
208 - 10. j.
208 - 11. f.
208 - 12. k.

209 - 1. b.
209 - 2. a.
209 - 3. b.
209 - 4. e.
209 - 5. c.
209 - 6. b.
209 - 7. b.
209 - 8. b.
209 - 9. a.
209 - 10. a.
109 - 11. f.

CHAPTER 2

210 - 1. Bile.
210 - 2. Store and concentrate bile.
210 - 3. Bile performs a digestive function in the emulsification of fats and activation of certain enzymes. It also functions in an excretory capacity, particularly with regard to cholesterol.
210 - 4. Bilirubin is normally reduced to urobilinogen in the intestines.
210 - 5. Bile is green due to biliverdin.
210 - 6. Although less urobilinogen is available for reabsorption in the intestines, liver damage may be severe enough to interfere with the uptake and transformation of urobilinogen in the liver. Hence, it appears in the urine in abnormal amounts.
210 - 7. Hemoglobin.
210 - 8. It is formed in the reticuloendothelial system and is conjugated with glucuronic acid in the liver to produce direct bilirubin.

211 - 1. Colesterol is formed from acetate ions in the liver and excreted as cholic acid in the bile.
211 - 2. Cholesterol esters are formed from organic acids in combination with the hydroxyl group of the cholesterol molecule.
211 - 3. 75 percent.
211 - 4. Lower.
211 - 5. F. Insoluble.
211 - 6. T.
211 - 7. F. 90 percent.
211 - 8. T.
211 - 9. T.
211 - 10. F. None.
211 - 11. T.
211 - 12. F. All hormones are derived from cholesterol.
211 - 13. T.
211 - 14. T.
211 - 15. F. Fat.
211 - 16. T.
211 - 17. F. It is not.
211 - 18. T.

212 - 1. Methyl alcohol, 40-50 percent.
212 - 2. a. Concentration of alcohol often causes some turbidity that is not always the same in the diazotized sample as in the serum blank.
  b. More than traces of hemoglobin present interferes with the color reaction.
  c. Color changes sensitive to the pH due to lack of sufficient buffer in reaction.

212 - 3. A solution containing relatively high concentration of caffeine and sodium benzoate is used to dissolve the free bilirubin for a reaction.
212 - 4. Buffers the pH of the diazo reaction.
212 - 5. a. Insensitive to pH.
  b. High diazo color.
  c. Stable color.
  d. Insensitive to hemoglobin concentration up to 750 mg per dl.

213 - 1. Cholesterol, acetic anhydride, glacial acetic acid, and sulfuric acid.
213 - 2. Cholesterol, sulfuric acid, acetic anhydride, and sulfosalicylic acid.
213 - 3. a. Bilirubin interference.
  b. Complexitieis of color reaction.
213 - 4. a. High bilirubin.
  b. Failure to mix when indicated or mixing when not indicated.
  c. Failure to adhere to order of addition of reagents.

214 - 1. a.
214 - 2. a.
214 - 3. b.
214 - 4. b.
214 - 5. c.
214 - 6. b.
214 - 7. c.
214 - 8. c.

CHAPTER 3

215 - 1. d.
215 - 2. e.
215 - 3. e.
215 - 4. f.
215 - 5. a.
215 - 6. b.
215 - 7. a.
215 - 8. g.
215 - 9. i.
215 - 10. h.

216 - 1. c.
216 - 2. b.
216 - 3. a.
216 - 4. d.
216 - 5. f.
216 - 6. e.
216 - 7. g.
216 - 8. h.
216 - 9. j.
216 - 10. i.

217 - 1. b.
217 - 2. a.
217 - 3. c.
217 - 4. e.
217 - 5. d.
217 - 6. f.
217 - 7. g.
217 - 8. g.
217 - 9. h.
217 - 10. h.

218 - 1. F. Lowest values.
218 - 2. F. Buiret reagent.
218 - 3. F. In urine.
218 - 4. T.

218 - 5. T.
218 - 6. T.

219 - 1. Electrophoresis.
219 - 2. Diagnosis of liver disease, congenital anomalies, and malignancies.
219 - 3. Sodium sulfite results in a more accurate and complete precipitation of globulin which agrees with electrophoretic patterns. Second, Na_2SO_3 is easier to work with because of its higher solution level, and buffering power.
219 - 4. To cause the globulin to form a mat.
219 - 5. Ammonium sulfate.
219 - 6. Na_2SO_3.
219 - 7. Protein nitrogen is measured and is an index to the amount of protein present.
219 - 8. An EDTA buffer to stabilize the dye using 2-(4 hydroxyazobenzene) benzoic acid (HABA).
219 - 9. Hemoglobin, red cell protein, and plasma protein.
219 - 10. 5 percent.
219 - 11. 0.15 to 0.30 g-%.
219 - 12. Hemoglobin.

CHAPTER 4

220 - 1. c, e.
220 - 2. c.
220 - 3. d.
220 - 4. f.
220 - 5. f.
220 - 6. a.
220 - 7. b.
220 - 8. g.
220 - 9. e.
220 - 10. j.
220 - 11. i.
220 - 12. h.
220 - 13. k.
220 - 14. m.
220 - 15. l.

221 - 1. e.
221 - 2. b.
221 - 3. a.
221 - 4. f.
221 - 5. g.
221 - 6. g.
221 - 7. h.
221 - 8. i.

222 - 1. b.
222 - 2. a.
222 - 3. a.
222 - 4. e.
222 - 5. c.
222 - 6. d.
222 - 7. f.
222 - 8. g.
222 - 9. h.
222 - 10. i.

223 - 1. e.
223 - 2. a.
223 - 3. d.
223 - 4. b.
223 - 5. c.
223 - 6. a.
223 - 7. c.
223 - 8. b.
223 - 9. c.
223 - 10. b.
223 - 11. c.
223 - 12. b.

224 - 1. Electrophoresis and paper chromatography.
224 - 2. Deproteinization.
224 - 3. A postprandial test is one performed after a meal.
224 - 4. *a.* In a 2-hour postprandial:
(1) Conditions of the test, such as patient activity, are better controlled.
(2) The carbohydrate intake is assured to be adequate.
(3) Subtle responses can be detected which may be a clinical necessity.
(4) Several results are better than one result.
b. Glucose toelrance tests are:
(1) Time-consuming for the laboratory.
(2) Clinically unnecessary when the postprandial is adequate.
(3) Inconvenient for the patient.
(4) Expensive in terms of time and reagents.

CHAPTER 5

225 - 1. They control the speed of biochemical reactions without reacting or undergoing change.
225 - 2. Units of activity rather than concentration.
225 - 3. 1μM (micromole) of substrate per liter of serum under specified condition of pH and temperature.
225 - 4. It makes far better comparison between methods and when converted into international units, the ranges are essentially the same.
225 - 5. The small number of substrate molecules decreases the chances that a molecule "hits" the bending site of the enzyme (E).
225 - 6. The activity of any enzyme varies directly with its concentration to a point at which further increase in enzyme produces no noticeable change in the speed of the reaction.
225 - 7. Substrate.
225 - 8. Inactivate and destroy it.
225 - 9. At 5° Centigrade on quick-freeze methods. Ice formation may inactivate the enzyme at slow freezing.
225 - 10. A 1° fluctuation can introduce an error of 10 percent plus or minus.
225 - 11. By a buffered substrate.
225 - 12. In addition to the probability of bacterial contamination of the specimen, the transaminose may be damaged by radiant energy.
225 - 13. Inhibit enzyme activity.
225 - 14. Enzymes are sometimes named for the substrate or for the function they perform.

226 - 1. b.
226 - 2. b.
226 - 3. d.
226 - 4. c.
226 - 5. e.
226 - 6. g.
226 - 7. i.
226 - 8. k.
226 - 9. m.
226 - 10. n.
226 - 11. a.
226 - 12. a.
226 - 13. p.
226 - 14. q.
226 - 15. a.

227 - 1. h, i.
227 - 2. f.
227 - 3. d.
227 - 4. g.
227 - 5. c.
227 - 6. a, b.
227 - 7. e.
227 - 8. j.

228 - 1. Liver dysfunction and bone diseases.
228 - 2. Prostatitis or prostate involvement, usually carcinoma of the prostate.
228 - 3. a. 4.9-5.1
b. 9.2-9.7
228 - 4. Carcinoma of the breast.
228 - 5. Serum is incubated with buffered glycerophospate of a definite alkaline or acid pH for 1 hour at 37° C.
228 - 6. Buffered p-nitrophenyl phosphate yields p-nitrophenol upon hyrdolysis. The p-nitrophenyl becomes yellow upon addition of NaOH and can be assayed spectrophotometrically.
228 - 7. The palsma is not suitable. Oxalates inhibit phosphatase activity.
228 - 8. Phenyl phosphate, β-glycerophosphate and p-nitrophenyl phosphate.

228 - 9. Malignant or granulomatous disease involving liver and hepatic cirrhosis, obstructive and hepatic jaundice.

229 - 1. a. For myocardial infarction.
b. For liver disorders.
229 - 2. This is to be expected; SGOT values are nearly always higher than SGPT values on a particular patient except in cases of viral hepatitis.
229 - 3. Activity that exceeds 350 units; 1:5 dilution with saline.
229 - 4. Will produce elevated results.
229 - 5. With refrigeration, LDH is very stable for 7 days.
229 - 6. Oxalate inhibits LDH.
229 - 7. Its elevation is much more prolonged, and is of special value if initial blood samples cannot be obtained at the time of the early infarction.
229 - 8. At a temperature range of 0°-4° C; never frozen.
229 - 9. Within 24 hours.
229 - 10. Investigation of skeletal muscle disease and myocardial infarction.
229 - 11. Elevates result due to muscle damage.
229 - 12. Elevates levels of CPK.
229 - 13. Store at 0°-4° C and ship frozen.
229 - 14. Lower results. They inhibit CPK activity.

230 - 1. T.
230 - 2. F. Leucine.
230 - 3. T.
230 - 4. F. It is not.
230 - 5. T.
230 - 6. T.
230 - 7. F. 6-phosphogluconolactone immediately hydrolyzes to 6-phosphogluconate.
230 - 8. T.
230 - 9. F. Regeneration of NADPH.
230 - 10. F. Greater than 45 percent.
230 - 11. F. It is specific and can be performed on stored blood.
230 - 12. F. Reduction of NADP to NADPH.
230 - 13. T.
230 - 14. F. Are not stable for more than few hours at room temperature or 4° C.

231 - 1. b.
231 - 2. b.
231 - 3. a.
231 - 4. a.
231 - 5. b.
231 - 6. a.

232 - 1. Enzymes existing in a variety of molecular forms which differ in their physical and chemical properties but show similar substrate specificity.
232 - 2. Electrophoresis.
232 - 3. Isoenzyme determination permits more precise recognition of their tissue of origin.
232 - 4. According to their electrophoretic mobility; LD_1, LD_2, LD_3, LD_4, and LD_5.

233 - 1. b.
233 - 2. a.
233 - 3. i.
233 - 4. h.
233 - 5. d, g.
233 - 6. c.
233 - 7. d.
233 - 8. c.
233 - 9. e.
233 - 10. f.
233 - 11. f.
233 - 12. f.

234 - 1. F. Pyridine nucleotides (NAD $\longrightarrow$ $NADH_2$.)
234 - 2. T.
234 - 3. F. 1 minute.
234 - 4. F. 25° C to 37° C.
234 - 5. F. Most advanced.
234 - 6. T.
234 - 7. T.
234 - 8. T.

STOP-

1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.

2. USE NUMBER 2 PENCIL ONLY.

90411 02 23

EXTENSION COURSE INSTITUTE
VOLUME REVIEW EXERCISE

LABORATORY PROCEDURES IN CLINICAL CHEMISTRY (PART I)

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that item numbers on answer sheet are sequential in each column.
3. Use a medium sharp #2 black lead pencil for marking answer sheet.
4. Write the correct answer in the margin at the left of the item. (When you review for the course examination, you can cover your answers with a strip of paper and then check your review answers against your original choices.) After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if a all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet-for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DONT'S:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than a #2 black lead pencil.

NOTE: NUMBERED LEARNING OBJECTIVE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Learning Objective Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Learning Objectives* indicated by these *Numbers*. The VRE results will be sent to you on a postcard which will list the *actual* VRE *items you missed.* Go to the VRE booklet and locate the *Learning Objective Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (200) It is clinically important to maintain the pH of blood within the range of

   a. 6.80 to 7.20.
   b. 7.20 to 7.36.
   c. 7.35 to 7.45.
   d. 7.40 to 7.53.

2. (200) The cations most frequently measured in the clinical laboratory are

   a. sodium and potassium.
   b. chloride and bicarbonate.
   c. proteins.
   d. carbohydrates.

3. (200) Water and electrolytes otherwise lost are normally conserved by the

   a. bladder.
   b. pancreas.
   c. kidneys.
   d. liver.

4. (201) Sodium has a primary function in

   a. osmotic equilibrium.
   b. cell permeability.
   c. muscle irritability.
   d. all of the above.

5. (201) Elevated plasma potassium (hyperkalemia) is associated with diseases of the

   a. kidneys and central nervous system.
   b. heart and central nervous system.
   c. thyroid and heart.
   d. stomach and kidneys.

6. (201) In the presence of a nondiffusible protein anion, the concentration of the diffusible sodium cation is

   a. less than that of the diffusible chloride anion.
   b. the same as that of the diffusible chloride anion.
   c. higher than that of the diffusible chloride anion.
   d. less than that of the nondiffusible protein.

7. (201) When chloride loss exceeds sodium loss, electrolyte balance is maintained by

   a. a decrease of plasma chloride.
   b. an increase of plasma chloride.
   c. a decrease of serum bicarbonate.
   d. an elevation of serum bicarbonate.

8. (202) The normal values for serum chloride, as compared to those of spinal fluid chloride, are

   a. essentially the same.
   b. lower.
   c. higher.
   d. variable.

9. (202) In the pilocarpine iontophoresis determination for early diagnosis of children with cystic fibrosis, a sweat chloride level

   a. above 60 mEq/L is indicative of an abnormal value.
   b. below 60 mEq/L is diagnostic for cystic fibrosis.
   c. of from 40 to 60 mEq/L is reported for homozygous individuals.
   d. of from 20 to 40 mEq/L is reported for heterozygous individuals.

10. (203) To maintain a pH of 7.4, which of the following ratios must be maintained?

a. 1:20 of bicarbonate to carbonic acid.
b. 20:1 of carbonic acid to bicarbonate.
c. 1:20 of carbon dioxide to carbonic acid.
d. 20:1 of bicarbonate to carbonic acid.

11. (204) In the Henderson-Hasselbalch equation, the pCO_2 is proportional to

a. carbonic acid or dissolved HCO_3.
b. bicarbonate or pH.
c. carbonic acid or dissolved CO_2.
d. bicarbonate or dissolved CO_2.

12. (204) Blood specimens for CO_2 content and direct pH measurements must be drawn anerobically because

a. carbon dioxide will diffuse to any other phase which has a lower partial pressure and is exposed to plasma.
b. carbon dioxide will diffuse to any other phase which has a higher partial pressure and is exposed to plasma.
c. carbonic acid will diffuse to any other phase which has a higher pressure and is exposed to plasma.
d. carbonic acid will diffuse to any other phase which has a lower pressure and is exposed to plasma.

13. (205) A patient with an abnormal pCO_2 (respiratory involvement) must have his acid-base problem evaluated on the basis of all of the following except

a. pH.
b. CO_2 combining power.
c. CO_2 content.
d. pCO_2.

14. (205) The purpose of the chloride shift is to

a. maintain electrical neutrality.
b. change the ratio of carbonic acid in the plasma.
c. assess acid-base equilibrium.
d. assess the blood-buffering capacity.

15. (205) What results may be expected if a patient loses CO_2 by hyperventilation?

a. An incerase in chloride and increase in bicarbonate.
b. A decrease in chloride and decrease in bicarbonate.
c. An increase in chloride and decrease in bicarbonate.
d. A decrease in chloride and decrease in bicarbonate.

16. (205) There is a decrease in bicarbonate in metabolic acidosis. This may occur as a result of

a. hyperventilation.
b. intestinal obstruction.
c. respiratory disease.
d. renal disease or diabetes mellitus.

17. (206) The standard electrode upon which all others are based is the

a. glass.
b. calomel.
c. hydrogen.
d. quinhydrone.

18. (207) A common clinical sympton of calcium deficiency is

a. tetanus.
b. tetany.
c. hyperparathyroidism.
d. hypokalemia.

19. (207) Which of the methods for calcium determination would not be affected by EDTA?

a. Titration.
b. Colorimetric.
c. Atomic absorption.
d. Oxalate precipitation.

20. (208) The serum level of phosphorus is closely related to the level of

a. arsenate.
b. silicate.
c. sulfate.
d. calcium.

21. (208) In diabetic acidosis, which of the following changes occurs?

a. Inorganic phosphorus level increases.
b. Inorganic phosphorus level decreases.
c. Calcium level increases.
d. Calcium level is not affected.

22. (208) The use of heparinized plasma is not recommended for inorganic phosphate determination because of the presence of

a. reducing substances in the commercial preparation of heparin.
b. phosphate ions in commercial preparations of heparin.
c. reducing substances in inorganic phosphate that react with heparin.
d. a substance that will precipitate the inorganic phosphate.

23. (209) Magnesium is usually determined by

a. colorimetry.
b. fluorometry.
c. flame photometry.
d. atomic absorption spectroscopy.

24. (209) Which of the following determinations is most likely to be requested in maintaining therapeutic levels in patients with psychiatric disorders?

a. Calcium.
b. Lithium.
c. Magnesium.
d. Phosphorus.

25. (209) All of the following statements concerning magnesium are true except that it

a. governs neuromuscular irritability.
b. is important for coenzymes in the metabolism of carbohydrates and proteins.
c. shows increased levels in malabsorption.
d. shows decreased levels in malabsorption.

26. (210) The major functions of the liver include all the following except

a. detoxification of harmful substances.
b. storage of glycogen and metabolic intermediates.
c. secretion of substances such as bilirubin conjugates, cholesterol, and dyes.
d. formation of blood constituents such as thrombin and production of red blood cells.

27. (210) The liver of an adult normally secretes how many ml of bile in 24 hours?

a. 300 to 500 ml.
b. 500 to 600 ml.
c. 400 to 500 ml.
d. 400 to 600 ml.

28. (210) Which of the following is not found in the feces of a normal healthy individual?

a. Bilirubin.
b. Urobilinogen.
c. Mesobilinogen.
d. Stercobilinogen.

29. (210) In complete extrahepatic obstruction, you would expect an increase in

a. intestinal bile.
b. direct and indirect serum bilirubin.
c. urine urobilinogen.
d. direct-reacting serum bilirubin only.

30. (210) The clinical laboratory is primarily concerned with all of the bile pigments except

a. urobilin.
b. urobilinogen.
c. stercobilin.
d. serum bilirubin.

31. (210) In liver cell degeneration, serum bilirubin is high and

a. bilirubin appears in the urine.
b. serum bilirubin is low.
c. intestinal urobilinogen is increased.
d. urine urobilinogen is decreased.

32. (210) Bile pigments are derived from the breakdown of

a. bilirubin.
b. iron.
c. hemoglobin.
d. urobilinogen.

33. (211) Cholesterol is a sterol characterized by the cyclic structure of the

a. open chain.
b. phenanthrene ring.
c. double bond.
d. tetracyclic ring.

34. (211) What percent of the cholesterol in the body is in the form of esters?

a. 25 percent.
b. 50 percent.
c. 55 percent.
d. 75 percent.

35. (211) Which of the following statements concerning the functions of cholesterol is incorrect?

a. Cholesterol level is increased following liver cell damage.
b. Cholesterol forms a large portion of brain tissue.
c. Cholesterol provides for formation of adrenal hormones.
d. Cholesterol functions in the transportation of fats.

36. (212) Which of the following is not a disadvantage in the Malloy-Evelyn method for determination of bilirubin?

a. Concentration of alcohol can cause turbidity.
b. Hemoglobin in more than trace amounts interferes with color reaction.
c. Color changes are sensitive to pH because of insufficient buffer in reaction.
d. Bilirubin combines with diazonium to form azo dyes.

37. (212) Which of the following statements is correct concerning the comparative chemical reactions of free bilirubin and bilirubin conjugates?

a. Free bilirubin is less soluble in water and reacts slowly or not at all in simple aqueous solution.
b. Free bilirubin is less soluble in water but reacts rapidly in an aqueous solution.
c. Free bilirubin is the conjugated form and is more soluble in water.
d. Free bilirubin is the direct-reacting bilirubin and is more soluble in an aqueous solution.

38. (212) In the Jendrassik and Frof method for determining bilirubin, the sodium acetate serves

a. as a color stabilizer for the diazo reaction.
b. as a pH buffer in the diazo reaction.
c. to accelerate the coupling of bilirubin with diazotized sulfanilic acid.
d. to neutralize the caffeine solution.

39. (213) All of the following are essential components of the Lieberman-Bouchard reaction except

a. acetic acid.
b. sulfuric acid.
c. ferric chloride.
d. acetic anhydride.

40. (213) Which of the following is not considered a source of error in the Schoenheimer-Sperry cholesterol procedures?

a. High bilirubin or hemolyzed specimen.
b. Failure to mix when indicated or mixing when the step is contraindicated.
c. Addition of reagents in reverse order.
d. Using 12 percent sulfosalicylic acid.

41. (214) If the thymol barbital buffer of the thymol turbidity test does not have a pH of 7.55, using a pH of 7.8 will result in values

a. 20 percent lower.
b. 30 percent lower.
c. 40 percent lower.
d. 50 percent lower.

42. (214) For every kilogram of body weight, the amount of BSP dye to inject in a test for liver function is

a. 1 mg.
b. 5 mg.
c. 1 ml of 5 percent.
d. 5 ml of 5 percent.

43. (215) The general formula for an alpha amino acid is

a. $R-CH(NH_2)-COOH$.
b. CH_3CHO.
c. $(CH_3)_4NOH$.
d. ROH.

44. (215) If a protein is in a salt solution of optional concentration at its isoelectric point, the protein molecules will

a. migrate.
b. fractionate.
c. denature.
d. precipitate.

45. (215) An example of a simple protein is

a. globulin.
b. mucoprotein.
c. phosphoprotein.
d. hemoglobin.

46. (216) What enzyme breaks down proteins in the stomach?

a. Peptidase.
b. Amylase.
c. Pepsin.
d. Trypsin.

47. (216) Which of the following amino acids can be produced by the body?

a. Threonine.
b. Valine.
c. Leucine.
d. Serotonin.

48. (216) A nucleic acid associated with factors of hereditary control is

a. PKU.
b. DNA.
c. HIAA.
d. RNA.

49. (217) In the biuret reaction, protein is reacted with an alkaline

a. sodium sulfate.
b. sodium sulfite.
c. copper sulfate.
d. copper sulfite.

50. (217) The Pandy test for CSF globulin is best described as

a. unreliable.
b. unstable.
c. acceptable.
d. desirable.

51. (217) Trichloroacetic acid is substituted for sulfosalicylic acid in the precipitation of urine protein because

a. trichloroacetic acid is more stable.
b. sulfosalicylic acid tends to give much more turbidity.
c. trichloroacetic acid tends to give much more turbidity.
d. trichloroacetic acid gives an even turbidity of albumin and globulin.

52. (217) The use of the standard protein "sticks" for screening spinal fluid protein might be considered ideal except that

a. CSF protein will not react with the tetrabromphenol blue indicator on the stick.
b. CSF protein is too sensitive to the phenol indicator on the stick.
c. strip indicator, tetrabromphenol blue, is less sensitive to globulins, which make up 50 percent of spinal fluid proteins.
d. the strip indicator, tetrabromphenol blue, is more sensitive to levels of globulin from 50 to 100 mg-%.

53. (219) Which of the following is not an advantage of using sodium sulfite rather than sodium sulfate in precipitating or "salting out" proteins from solution?

a. Will not crystallize out at refrigerator temperature.
b. Yields albumin and globulin more consistent with electrophoretic measurements.
c. Will remain in solution at room temperature.
d. Has a better buffering power.

54. (219) Which of the following procedures for the estimation of fibrinogen is obsolete?

a. Use of a neutral salt (12.5 percent Na_2SO_3).
b. Quantifying fibrinogen on the basis of electrophoretic mobility.
c. Mixing 1 ml of freshly drawn blood with 0.1 ml topical thrombin.
d. Recovery of fibrin, the amount of which is dependent upon the amount of both fibrinogen and prothrombin.

55. (219) The normal value for total protein per 100 ml of serum is

a. 4 to 6 mg.
b. 6 to 8 mg.
c. 4 to 6 g.
d. 6 to 8 g.

56. (219) The molecular weight of hemoglobin in atomic mass units is

a. 6,450 amu.
b. 10,000 amu.
c. 64,500 amu.
d. 100,000 amu.

57. (220) Monosaccharides differ from disaccharides in that monosaccharides

a. do not hydrolyze into other sugars.
b. are classified according to the number of carbon atoms they contain.
c. yield two molecules of a simple sugar upon hydrolysis.
d. become polysaccharides upon hydrolysis.

58. (220) Glucose is also called

a. dextrin.
b. dextrose.
c. dextran.
d. sucrose.

59. (220) The two common forms of stereoisomerism are

a. spacial and atomic.
b. double bond and structural.
c. geometric and optical.
d. asymmetric and symmetric.

60. (220) Polarized light is defined as light which

a. vibrates in only one plane.
b. passes through a crystal.
c. correlates with optical rotation.
d. consists exclusively of ultraviolet rays.

61. (221) In what chemical form are carbohydrates absorbed in the intestine?

a. Polysaccharides.
b. Disaccharides.
c. Glyceraldehyde.
d. Monosaccharides.

62. (221) Glycolysis takes place in

a. the liver only.
b. the muscles only.
c. both the liver and in the muscles.
d. neither the muscles nor in the liver.

63. (221) Which of the following terms may be used to designate the series of steps involved in the aerobic metabolism of glucose?

a. Krebs cycle.
b. Citric-acid cycle.
c. TCA cycle.
d. Any of the above.

64. (222) The true glucose level of blood is

a. 60 to 80 mg-%.
b. 70 to 100 mg-%.
c. 80 to 120 mg-%.
d. 90 to 130 mg-%.

65. (222) Insulin regulates all of the following except the

a. rate of glucose utilization.
b. rate of glucose output from the liver.
c. rate of glucose breakdown in the liver.
d. transfer of glucose across cell membranes.

66. (222) In the glucose tolerance test, which of the following conditions indicate a normal response to glucose intake?

a. Fasting specimen normal; 1-hour specimen less than 180 mg/dl; 2-hour specimen less than 140 mg/dl; and all collected urine specimens negative.
b. Fasting specimen normal; 1-hour specimen less than 180 mg/dl; 2-hour specimen greater than 140 mg/dl; and all collected urine specimens negative.
c. Fasting specimen normal; 1-hour specimen less than 180 mg/dl; 2-hour specimen less than 140 mg/dl; and all collected urine specimens except the last negative.
d. Fasting specimen normal; 1-hour specimen greater than 180 mg/dl; 2-hour specimen less than 140 mg/dl; and all collected urine specimens negative.

67. (223) In the ortho-toluidine procedure for glucose, what stabilizing agent is now used?

a. Ortho-toluidine.
b. Thiourea.
c. Glacial acetic acid.
d. Peroxidase.

68. (223) As applied to the measurement of blood glucose, the Folin-Wu method is

a. generally unacceptable.
b. the best method to use.
c. generally acceptable.
d. too specific.

69. (223) The precision of the ortho-toluidine procedure for glucose is ±5 percent for glucose values between

a. 80 and 120 mg/100 ml.
b. 70 and 100 mg/100 ml.
c. 50 and 300 mg/100 ml.
d. 50 and 150 mg/100 ml.

70. (223) In the Somogyi-Nelson true glucose procedure, alkaline copper solution is heated with a specimen filtrate which

a. reduces cupric oxide.
b. reduces zinc hydroxide.
c. oxidizes arsenomolybdate.
d. reduces cupric hydroxide.

71. (223) In the glucose oxidase procedure, glucose is converted to gluconic acid and

a. hydrogen peroxide.
b. water.
c. peroxidase.
d. o-dianisidine.

72. (224) The so-called PMS reagent for determining glucose contains a solution of

a. phenylphthalein in methanol.
b. phenol in methyl cellusolve.
c. phenol in methly salicylate.
d. phenol, methanol, and salicylic acid.

73. (224) Which of the following statements concerning the report by the American Diabetes Association Committee on Statistics on glucose tolerance tests is not true?

a. Patients should ingest at least 150 grams of carbohydrate daily for at least 3 days prior to the test.
b. The test should not be done during hospitalization of acutely ill patients with decreased dietary intakes.
c. Guidelines for interpretation of results are based on values obtained in the ambulatory patients.
d. Guidelines for interpretation of results are based on values obtained from random samples of sick and well patients.

74. (224) Which of the following statements concerning the oral glucose tolerance test is incorrect?

a. The patient should avoid vigorous exercise.
b. The patient may drink moderate amounts of water.
c. The patient should not eat during the test.
d. The patient may eat during the test if he is hungry.

75. (225) Enzymes are best described as

a. phospholipids.
b. biocatalysts.
c. mucoproteins.
d. nucleoproteins.

76. (225) Which of the following statements concerning international units (IU) is correct?

a. A unit is 1 μM of substrate used per minute per liter of serum under specified conditions of pH and temperature.
b. International units are generally used in the clinical laboratory.
c. The use of international units does not provide for good comparison between methods.
d. A unit is 1 μM of enzyme used per minute per liter of serum under specified conditions of pH and temperature.

77. (225) The enzyme activity is slower in a substrate of low concentration because

a. the small number of substrate molecules increases the chances that a molecule will "hit" the binding site of the enzyme.
b. the small number of substrate molecules decreases the chances that a molecule will "hit" the binding site of the enzyme.
c. the reduced number of substrate molecules decreases the rate of substrate reaction.
d. the small number of enzyme molecules decreases the chances that a molecule will "hit" the binding site of the enzyme.

78. (225) The activity of any enzyme will approximately double for every temperature increase on the centigrade scale of

a. 1°.
b. 2°.
c. 5°.
d. 10°.

79. (225) The "active site" of the substrate molecules is the site at which the

a. substrate is bound.
b. substrate is neutralized.
c. enzyme neutralizes the substrate.
d. substrate is destroyed.

80. (225) The period of time during which an enzyme acts determines the

a. amount of enzyme used.
b. amount of substrate converted.
c. clinical value of the result.
d. enzyme concentration.

81. (226) Amylase catalyze the reaction by which

a. maltose is hydrolyzed into dextrose and dextrain.
b. maltose is hydrolyzed into lactose and dextrose.
c. starch is hydrolyzed into maltose and dextrose.
d. dextrose is hydrolyzed into maltose and lactose.

82. (226) If serum is hemolyzed, lipase levels are known to show a

a. significant increase.
b. slight increase.
c. significant decrease.
d. slight decrease.

83. (227) Which of the following enzyme tests would likely be requested when a myocardial infarction is suspected?

a. Cholinesterase.
b. Leucine aminopeptidase (LAP).
c. Lipase.
d. Creatine phosphokinase (CPK).

84. (227) Which of the following enzyme tests would be of diagnostic value in suspected cases of organo-phosphorus poisoning?

a. SGOT.
b. CPK.
c. Aldolase.
d. Cholinesterase.

85. (228) In the Shinowara, Jones, and Reinhart method for alkaline phosphatase, the serum is incubated with buffered

a. glycerophosphate.
b. phospholipid.
c. phosphatase.
d. nitrophosphate.

86. (228) In the Bessy, Lowry, and Brock method for alkaline phosphatase, the substrate used is

a. -naphthyl phosphate.
b. phenyl phosphate.
c. buffered p-nitrophenyl phosphate.
d. -glycerophosphate.

87. (228) 5'-nucleotidase level shows a similar trend of increased values in both obstructive and hepatic jaundice when compared to

a. acid phosphatase values.
b. alkaline phosphatase values.
c. lactic dehydrogenase values.
d. CPK values.

88. (229) SGPT values are higher than SGOT values in

a. alcoholic hepatitis.
b. obstructive jaundice.
c. viral hepatitis.
d. all of the above.

89. (229) When separated from cells, the LDH in serum is stable for up to how many days with refrigeration?

a. 7.
b. 10.
c. 15.
d. 20.

90. (229) Hemolyzed specimens used for SGOT and SGPT determinations cause values to be

a. decreased.
b. increased.
c. slightly decreased.
d. unaffected.

91. (229) Sources of error in CPK determinations do not include

a. variations in temperature.
b. appreciable hemolysis.
c. intramuscular injection.
d. the use of serum instead of plasma.

92. (230) If stored at 4° C, leucine aminopeptidase (LAP) is stable in urine for approximately

a. 30 days.
b. 20 days.
c. 14 days.
d. 7 days.

93. (230) Glucose-6-phosphate dehydrogenase is found principally in

a. red blood cells.
b. serum.
c. urine.
d. white blood cells.

94. (230) A deficiency of G-6-PD results in a limitation of the regeneration of

a. DPN.
b. DPNH.
c. NAD.
d. NADPH.

95. (230) G-6-PD is stable in erythrocytes for 3.5 weeks at 30° C when preserved with which of the following substances?

a. Heparin.
b. Glucose-EDTA.
c. Alsever's solution.
d. EDTA.

96. (231) Which of the following constituents is least used in the determination of trypsin in duodenal contents?

a. Casein.
b. Sodium bicarbonate.
c. Duodenal fluid.
d. Acetic acid.

97. (232) Which statement concerning isoenzymes is not correct?

a. Isoenzymes are generally separated by serologic tests.
b. The enzyme of a single tissue may be separated into a number of isoenzyme fractions.
c. Isoenzymes are generally separated by electrophoresis.
d. Isoenzymes exist in a variety of molecular forms which differ in their physical and chemical properties but show similar substrate specificity.

98. (232) The isoenzymes of lactic dehydrogenase (LD) are designated in ordinary usage according to their

a. molecular structures.
b. physico-chemical properties.
c. electrophoretic mobility.
d. rates of catalyzing chemical reactions.

99. (233) Ornithine carbamyl transferase (OCT) occurs almost exclusively in cells of

a. heart tissue.
b. liver tissue.
c. striated muscle.
d. smooth muscle.

100. (233) Specimens for aldolase determination are stable for how many days in a frozen state?

a. 3.
b. 4.
c. 5.
d. 6.

101. (234) In the determination of lactic dehydrogenase using the UV procedure, the assessment of adherence to zero-order reaction is accomplished by multiple measurements at

a. 30-minute intervals.
b. 15-minute intervals.
c. 10-minute intervals.
d. 1-minute intervals.

102. (234) Most UV enzyme procedures depend upon the absorption at

a. 340 mμ (nm) of pyridene nucleotides.
b. 440 mμ (nm) of pyridene nucleotides.
c. 340 mμ (nm) of diphosphopyridene nucleotide (DPN).
d. 440 mμ (nm) of diphosphopyridene nucleotide (DPN).

103. (234) Which of the following temperature ranges is recommended for the determination of ultraviolet enzyme?

a. 13° C to 25° C.
b. 25° C to 37° C.
c. 37° C to 50° C.
d. 50° C to 62° C.

104. (234) Which of the following methods for enzyme determination provides best reliability when a measure of other biological fluid constituents is required?

a. Colorimetric method.
b. Electrophoretic method.
c. Ultraviolet endpoint method.
d. Ultraviolet kinetic method.

105. (234) In the colorimetric determination of SGOT in which the produced oxalacetate is measured, the procedure is considered nonspecific or inaccurate because

a. keto acids interfere with the determination and exalacetate inhibits the enzymatic reaction.
b. phenazine interferes with the determination and oxalacetate intensifies the enzymatic reaction.
c. keto acids interfere with the determination and exalacetate intensifies the enzymatic reaction.
d. phenazine interferes with the determination and oxalacetate inhibits the enzymatic reaction.

STUDENT REQUEST FOR ASSISTANCE

PRIVACY ACT STATEMENT

AUTHORITY: 44 USC 3101. **PRINCIPAL PURPOSE(S):** To provide student assistance as requested by individual students. **ROUTINE USES:** This form is shipped with every ECI course package. It is utilized by the student, as needed, to place an inquiry with ECI. **DISCLOSURE:** Voluntary. The information requested on this form is needed for expeditious handling of the student's need. Failure to provide all information would result in slower action or inability to provide assistance.

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1. THIS COURSE CONCERNS COURSE
2. TODAY'S DATE
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SECTION III: REQUEST FOR MATERIALS, RECORDS, OR SERVICE *(Place an 'X' through number in box to left of service requested.)*

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1	Extend Course Completion Date. *(Justify in Remarks)*
2	Send VRE Answer Sheets for Vol(s) *(Circle)*: 1 2 3 4 5 6 7 8 9. Originals were *(Circle one)* Not received; Lost; Misused.
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CDC 90411

MEDICAL LABORATORY TECHNICIAN (CHEMISTRY AND URINALYSIS)

(AFSC 90470)

Volume 3

Laboratory Procedures in Clinical Chemistry (Part II)

Extension Course Institute
Air University

PREPARED BY
SCHOOL OF HEALTH CARE SCIENCES, USAF (ATC)
SHEPPARD AIR FORCE BASE, TEXAS

EXTENSION COURSE INSTITUTE, GUNTER AIR FORCE STATION, ALABAMA

THIS PUBLICATION HAS BEEN REVIEWED AND APPROVED BY COMPETENT PERSONNEL
OF THE PREPARING COMMAND IN ACCORDANCE WITH CURRENT DIRECTIVES
ON DOCTRINE, POLICY, ESSENTIALITY, PROPRIETY, AND QUALITY.

Preface

BEFORE BEGINNING this volume you must have completed Volumes 1 and 2 of Career Development Course 90411. In the first two volumes you studied general principles related to clinical chemistry and several fundamental categories of routine laboratory tests.

In the present volume we will continue very much in the same way as in Volume 2. That is, categories of laboratory tests will be described with regard to physiological and chemical principles. The significant difference between Volume 2 and Volume 3 is that Volume 3 includes procedures which are performed more frequently in large facilities than in laboratories of Class C or below.

Chapter 1 of Volume 3 consists of two sections: "Retention and Excretion Tests" and "Clearance and Concentration-Dilution Tests." Some attention is devoted to the calculations involved in the various types of clearance tests. In Chapter 2, the subject of gastric analysis is discussed, which is somewhat unique in terms of the specimen involved and its relationship (or lack of it) to other areas of clinical chemistry. New dimensions in gastric analysis and reporting are discussed with current applications. After this brief chapter, we discuss the more complicated special chemistry tests.

There are two sections in Chapter 3: the first, "Referral Chemistries," includes chromatography, electrophoresis, and other tests generally performed only by consultant centers. The second part of Chapter 3 is a treatment of hormones.

Concluding this volume is a chapter concerned with toxicology from the standpoint of both the Class A laboratory and the smaller hospital laboratory.

If you have questions on the accuracy or currency of the subject matter of this text or recommendations for its improvement, send them to School of Health Care Sciences/MSTW, Sheppard AFB TX 76311. NOTE: Do not use the suggestion program to submit corrections for typographical or other errors.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Behavior Objective Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFS AL 36118, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 24 hours (8 points).

Material in this volume is technically accurate, adequate, and current as of May 1976.

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NOTE: In this volume, the subject matter is developed by a series of Learning Objectives. Each of these carries a 3-digit number and is in boldface type. Each sets a learning goal for you. The text that follows the objective gives you the information you need to reach that goal. The exercises following the information give you a check on your achievement. When you complete them, see if your answers match those in the back of this volume. If your response to an exercise is incorrect, review the objective and its text.

Kidney Function Tests

RENAL FUNCTION tests are generally influenced by both renal factors and extrarenal factors. To properly evaluate the functions of the kidney, you must understand its anatomy and physiology.

As will be described in greater detail in Volume 4, the functional unit of the kidney is the nephron unit, which is composed of the glomerulus and accompanying tubules. This unit selectively filters the blood which passes through it in order to eliminate or retain certain substances in the blood. At the same time, selective reabsorption and secretory activity occurs in the tubules, including retention of most of the water which passes through the nephron unit.

Methods that are reasonably specific for renal physiologic function and are common in the clinical laboratory are presented in this chapter. For the amount of time and effort required, few tests provide clinical information as definitive as kidney function tests.

No attempt has been made to classify the tests according to a particular function of the kidney (for example, tubular secretion), since some tests measure more than one function. You should be aware of the nature of kidney function tests from the standpoint of purpose, principle, and procedure.

1-1. Retention and Excretion Tests

Retention tests are a measurement of blood levels of such constituents as urea nitrogen to determine the extent to which they are being retained by the kidney. If we are trying to determine whether a substance is being eliminated from the blood, we perform an excretion test. For example, the PSP test is based on the excretion of a foreign substance (dye) by the kidney.

400. Name four major functions of the kidneys, three physiological factors that play a role in normal kidney activity, and four major categories of kidney function tests.

Purpose of Kidney Function Tests. Before discussing kidney function tests, let's review some of the major functions of the kidney.

It is the purpose of a kidney function test to evaluate one or possibly more of the following kidney functions: (1) maintenance of acid-base and electrolyte balance, (2) excretion of waste products of metabolism, (3) maintenance of osmotic equilibrium, and (4) excretion of foreign substances, for example, dyes, poisons or drugs. To properly evaluate the functions of the kidney, you must understand its anatomy and physiology.

The ability of the kidneys to function properly depends upon three major factors. They are (1) the rate of renal blood flow, (2) the activity of the kidney tubules, and (3) the rate and efficiency of glomerular filtration. Accordingly, kidney function tests are sometimes classified to provide an indication as to the site of impaired kidney function. For example, a dye excretion test is valuable in measuring renal blood flow. Activity of the tubules can be measured with concentration and dilution tests, while the glomerular filtration rate can be evaluated by clearance tests. In addition to ordering a test to determine the origin or extent of kidney disorders, the physician may be interested in avoiding clinical problems which arise from kidney malfunction. These problems include a buildup of urea and other waste products in the blood, the concentration of which must be determined.

Four categories of kidney function tests which are of particular interest to the clinical laboratory are clearance tests, excretion tests, concentration-dilution tests, and retention tests.

Exercises (400):

1. List four major functions of the kidneys.

2. List three major physiological factors which play a role in normal kidney activity.

3. List four major categories of kidney function tests.

401. Describe the procedures for determination of urea nitrogen in terms of methods, advantages and disadvantages of the methods, principles, interpretation of results, and possible sources of errors.

Urea Nitrogen. Urea nitrogen constitutes about 45 percent of the total nonprotein nitrogen (NPN) in serum and plasma. The determination of serum urea nitrogen is considered the most popular screening test for the evaluation of kidney function. Earlier biochemists were interested in the nitrogen balance, in which all blood and urine nitrogenous constituents were added together in terms of their nitrogenous content.

Urea, the major end product of protein metabolism in man, is derived principally from the amino groups of amino acids. The liver is considered to be the major organ capable of urea synthesis via the ornithine cycle. The molecular weight of urea ($NH_2.CO.NH_2$) is 60. The urea molecule contains two nitrogen atoms with a total weight of 28. Since the urea in the blood is often expressed as BUN (blood urea nitrogen), the conversion of urea nitrogen value to urea is performed by multiplying the BUN by $\frac{60}{28}$ or 2.14. Thus a BUN of 40 mg/dl is equal to 40 × 2.14 or 85.6 mg/dl. You may note that the concentration of BUN is approximately one-half that of urea.

Methods of determination. The methods used for determination of urea are classified into three groups:

a. Direct condensation of urea with diacetyl to form a measurable chromogen.

b. Indirect determination of ammonia as a product of urease action on urea.

c. Miscellaneous procedures involving various photometric or physical principles of analysis.

Although the determinations are most often performed on serum, the results are still conventionally reported as BUN. Concentrations of urea in whole blood, serum, or plasma are quite similar, and no significance can be implied to differences between blood and serum determinations.

Advantages and disadvantages. One important advantage of the direct method using diacetyl monoxime (also called 2,3 butanedione monoxime) is that it does not determine NH_3 (ammonia). Diacetyl monoxime reacts directly with urea as well as with dibasic amino acids and possibly with peptides that are present in insignificant quantities in normal blood. Manual methods, as well as methods adapted for the auto-analyzer, have been developed for this procedure.

While the diacetyl methods are widely used, some disadvantages should be kept in mind:

a. The color develops and fades rapidly.

b. The color is photosensitive.

c. The color does not follow Beer's law with either a filter photometer or a spectrophotometer.

d. The unpleasant odor and evident fumes make it advisable to work in a fume hood.

e. With diacetyl monoxime, the time of heating for maximal color development is dependent on the urea concentration.

f. The reaction is not completely specific.

Method of Gentzkow and Masen. The indirect method of Gentzkow and Masen uses the enzyme urease to hydrolyze urea to ammonium carbonate. The ammonium carbonate produced is then nesslerized and compared photometrically with a suitable standard. Nessler's reagent is made up of the following reagents:

Mercuric iodide (HgI_2)	45.5 g
Potassium iodide (KI)	34.9 g
Potassium hydroxide (KOH), 10 M	200.0 ml
Distilled water, q.s.	1000.0 ml

Berthollet's reaction. Even though the nesslerization techniques are still widely used, the methods employing the indophenol reaction of Berthollet are becoming increasingly popular because of their greater sensitivity. The Berthollet's reaction is about 10 times more sensitive for ammonia than nesslerization. Serum urea is hydrolyzed with urease, and the released ammonia then is measured as indophenol. This method was once considered cumbersome until the four reagents were reduced to two: the catalyst-phenol solution and the alkaline-hypochlorite solution.

Method of Searcy et al. One such method by Searcy et al. is outlined in AFM 160-49, *Clinical Laboratory Procedures—Chemistry and Urinalysis.* Urea in the specimen is converted to ammonia by the enzyme urease in the buffered solution. Upon adding sodium phenate and hypochlorite, a stable blue color is developed with an intensity proportionate to the ammonia concentration. The reagents used are buffered urease, phenol color reagent, alkaline hypochlorite reagent and a nitrogen standard (0.15 mg/ml).

In the procedure, an unknown, a standard, and a blank are incubated in a 37° C water bath for 15 minutes to convert urea to ammonia. After incubation, add phenol color reagent to each tube and mix; then add the alkaline hypochlorite promptly after the mixing of the phenol reagent. Incubate the tubes again for 20 minutes at 37° C. Add water to the tubes and mix by inversion. Read the absorbance of the unknown, the standard, and the blank against distilled water.

Sources of error. Some of the sources of error include the following:

a. Anticoagulants containing ammonia must not be used in preparing specimens for analysis.

b. Reagents for the procedure will deteriorate rapidly if left at room temperature for any appreciable length of time.

c. Ammonia-free distilled water must be used in preparing all reagents.

d. The test procedure is highly sensitive for ammonia and should be free of ammonia fumes because ammonia will elevate test results.

e. Streptomycin and chloramphenicol may interfere with the action of the enzyme, giving low results.

f. Mercury compounds (for example, mercurial diuretics) will interfere both with the enzyme reaction and with the color formation, leading to low results.

Significance of elevated or lowered results. Elevated urea nitrogen values are found in conditions associated with impaired renal function, particularly chronic nephritis. Cases of acute nephritis, cardiac failure, prostatic obstruction, and intestinal obstruction may also cause the urea nitrogen to be elevated. Lowered urea nitrogen results may be encountered in impaired liver function and certain other conditions.

Any drug having marked diuretic effect may cause a lowering of the BUN; conversely, any drug having toxic effect on the kidneys will cause an increase of the BUN. After appropriate incubation, optical density values of test solutions will not change appreciably at room temperature for several hours. The presence of hemoglobin in the specimen does not significantly alter test results. Color reaction mixtures from samples containing more than 60 mg of urea nitrogen per dl should be diluted directly and read in conjunction with a reagent blank diluted in the same manner. Dilutions of three to five times with distilled water is recommended. The normal range for a BUN is 8-20 mg per dl of serum or plasma.

Exercises (401):

1. Methods for determination of urea are classified into what three groups?

2. How does the direct method using diacetyl monoxime determine BUN?

3. What are some disadvantages of the diacetyl methods?

4. What is the principle of the Gentzkow and Masen method for BUN determination?

5. What is the principle of Berthollet's indophenol method for BUN determination?

6. How would you expect an anticoagulant to affect the results of your determination (increase or decrease)?

7. What effect will antibiotics such as streptomycin and chloramphenicol have on the test?

8. What effect does the presence of hemoglobin have on the BUN results?

402. Describe the procedures for determinations of uric acid, creatinine, and creatine in terms of body production, normal levels, significance of abnormal levels, and principles of the methods.

Uric Acid. Occurring as the end product of purine metabolism, uric acid ($C_5H_4N_4O_3$) is a component of the total nonprotein nitrogen of the body. However, the blood uric acid level does not relate directly to the NPN level. Uric acid is normally excreted in the urine. The blood plasma level of uric acid depends upon both its rate of formation and the rate at which the kidneys are able to eliminate uric acid. Hence, elevated plasma uric acid levels are usually due to overproduction, decreased destruction, or decreased excretion.

Elevated levels. Diseases which contribute to one or more of these factors and which result in elevated levels include renal disorders, blood dyscrasias, lead poisoning, liver disease, and gout. Gout is a metabolic disease involving purine metabolism and characterized by marked inflammation of the joints. The plasma uric acid level may rise to 10 or 15 mg per dl during the acute phase of gout. Levels near the upper limit of normal—that is, 6 to 8 mg per dl—may be particularly difficult to interpret in view of technical difficulties sometimes encountered with the uric acid procedure. A quality control program is very helpful in this regard. Decreased values are of no known significance.

Method of Henry et al. One of the most common procedures for the determination of uric acid is the method of Henry et al. In this test, hexavalent phosphotungstic acid in alkaline solution is reduced by uric acid to a lower valence with the formation of a blue color. Sodium carbonate may be used as the source of alkali. The phosphotungstic acid reagent should be prepared in the following manner:

Sodium tungstate (Na_2WO_4), R.G.	40 g
Phosphoric acid (H_3PO_4), 85 percent	32 ml
Lithium sulfate ($Li_2SO_4 \cdot H_2O$)	32 g
Distilled water—q.s. to	1000 ml

Dissolve the sodium tungstate in 300 ml of distilled water in a reflux flask and add the phosphoric acid. Add several glass beads and reflux gently for 2 hours. Cool to room temperature and q.s. to 1000 ml with distilled water. Add the lithium sulfate. NOTE: Refluxing is an essential part of reagent preparation. Incorrect preparation of phosphotungstic acid reagent will probably result in turbidity of the color development mixture. Serum is the preferred specimen for analysis as it lacks the non-uric-acid constituents and color-producing agents that may be found in whole blood.

a. The normal values in serum are:
(1) Males: 2.0-8.0 mg per dl.
(2) Females: 1.0-7.0 mg per dl.
b. The normal values in urine for males and females are 250-750 mg per 24-hour volume.

Several commercially prepared reagent kits provide the time-saving convenience of having the reagents already prepared with a standard and ready for use. These are especially convenient in laboratories where refluxing apparatus is not readily available.

Creatinine. One of the nonprotein constituents of blood, creatinine, is also known chemically as methylglycocyamidine, $C_4H_7ON_3$. An elevation in the serum creatinine level *may* occur as a concomitant condition whenever the blood urea level is increased. This would be a likely occurrence in nephritis, urinary obstruction or suppression, cardiac decompensation, and intestinal obstruction. The assay of creatinine is of particular value when it is performed as a clearance test, which will be discussed in a following paragraph.

Jaffe reaction. A widely accepted chemical procedure involves the Jaffe reaction. The creatinine in a PFF reacts with picric acid in an alkaline solution to form creatinine picrate, which is yellow to red in color. A tungstic acid filtrate is suitable. There are a few precautions which must be observed. The specimen (serum or urine) should not contain dyes, for example, PSP or BSP. Thymol and toluene are suitable urinary preservatives, but acids and alkalis must not be used because they convert creatine to creatinine. Be careful not to confuse creatinine with *creatine*. Creatine is described in a paragraph below.

Precautions. The color development mixture should be read at room temperature because heat causes a deviation from Beer's law. Another precaution to be observed, in addition to those already mentioned, is in regard to the explosive nature of alkaline picrate. For this reason, alkaline picrate *must not* be heated in a dry-air oven to dehydrate it or for any other purpose. Alkaline picrate reagent is quite stable at room temperature if it is protected from light. It stains clothing and other objects and should, therefore, be handled carefully.

a. The normal values for creatinine in serum are:
(1) Males: 0.9-1.4 mg per dl.
(2) Females: 0.8-1.2 mg per dl.
b. The normal values in urine are:
(1) Males: 1.0-2.0 g per 24 hours.
(2) Females: 1.0-2.0 g per 24 hours.

Creatine. Creatine is described chemically as methylglycocyamine NH, the formula for which is $C(NH_2)N - (CH_3)CH_2COOH$. Unlike creatinine, creatine is not a waste product of metabolism, but is derived from glycine, arginine, and methionine. Creatine functions in muscle contraction in the form of phosphocreatine. Creatine is assayed from urine by measuring an increase in creatinine concentration upon heating and applying a conversion factor for differences in molecular weight. Conversion is accomplished with the addition of acid and application of heat to the specimen by boiling. Increased values are found in fever, malnutrition, pregnancy, and diseases associated with muscular weakness.

a. Creatine normals in serum are as follows:
(1) Males: 0.17-0.50 mg per dl.
(2) Females: 0.35-0.93 mg per dl.
b. Creatine normals in urine are:
(1) Males: Up to 150 mg per 24 hours.
(2) Females: Up to 250 mg per 24 hours.

Normal values for creatine are somewhat controversial, particularly since as much as 10 to 30 percent of the urinary creatine may be converted to creatinine in the bladder.

Exercises (402):

1. Where does uric acid come from in the body?

2. What does an elevated serum uric acid level indicate?

3. What is the principle of the phosphotungstic acid procedure for uric acid by the method of Henry, *et al.* as described in this text?

4. What purpose does the sodium carbonate serve in this procedure?

5. What is the normal serum uric acid level?

6. Of what clinical significance is a decreased serum uric acid?

7. What is the general chemical nature of creatinine?

8. What is the principle of the creatinine procedure involving the Jaffe reaction?

9. Distinguish between creatinnine and creatine.

10. How is creatine assayed?

403. Indicate the procedures for the PSP excretion test by telling when specimens should be collected, what substances might interfere with the test, and what factor prevents the patient from receiving an overdose of the dye.

PSP Excretion Test. Phenolsulfonphthalein (PSP, phenol red) is a dye which is readily removed from the blood and excreted by the normal, healthy kidney. Most of the dye is secreted by the kidney tubules, while a lesser amount is filtered by the glomeruli. A small amount is excreted by the liver into the bile. The rate of excretion of PSP depends also on the state of renal blood flow. The amount of dye excreted is, therefore, an indication of the excretory state of the kidneys, especially the tubules.

Injection of dye. PSP dye is supplied through medical supply channels in ampules containing 1 ml of dye, which is equivalent to 6 mg of PSP. This dye is transferred aseptically to a *sterile* syringe and injected intravenously by a physician. The laboratory technician frequently draws the dye into a syringe for use by the physician. It is good aseptic technique to attach a new sterile needle after the dye has been drawn into the syringe from the ampule and after air has been expelled from the syringe. It is also good aseptic technique to leave the protective sheath on the needle rather than place the sterile needle on a pad of cotton or gauze soaked with nonsterile alcohol. A potential source of error in this test is injecting too much dye and introducing an error in calculations. This is unlikely, however, since one would not normally use more than one ampule of dye per patient. The patient should drink water before the test. Whether the patient's bladder is empty or not at the beginning of the test is not particularly significant. Time of injection is noted and the patient is asked to empty his bladder completely at periods of exactly 15 minutes, 30 minutes, 1 hour, and 2 hours after injection of the dye.

Instruct the patient not to discard any urine and provide him or her with one container of adequate size or two regular size urine bottles for each specimen. After all four specimens have been collected, label each with the time of collection and the volume. It is also advisable to note the volume on the report submitted to the physician. Centrifuge or filter turbid urine before proceeding to the analysis.

Procedure. The entire volume in each container may be transferred to a 1-liter flask labeled in the same manner as the container. It is then alkalized with 3 ml of 4N NaOH, and q.s.'d with water. An alternate procedure is to transfer 1 percent of the urine volume to a flask and add 1 ml of 1 percent NaOH, followed by sufficient water to bring the volume to 10 ml. This actually amounts to reducing the volume of specimen by a factor of 100 and then reducing the dilution by a factor of 100. Both procedures accomplish the same result. The normality of the NaOH need not be exact.

Interpretation of results. Read each specimen in a spectrophotometer and compare it with a standard or prepared graph. There is usually no significant variation in readings of the standard; it is, therefore, quite acceptable to prepare a curve. The color is stable, and there are only a few technical precautions to observe. First, the test should not be run within 24 hours after a BSP test, because the BSP test may interfere slightly with the PSP test. Second, bile and hemoglobin in the specimen may interfere, but either may be extracted with a zinc acetate-methanol mixture. Normal kidneys excrete at least 25 percent of the dye in 15 minutes and a total of at least 65 percent of the dye in 2 hours. The percentage of dye should diminish with each successive urine specimen and, of course, should not total more than 100 percent for all four specimens.

Exercises (403):

1. After the PSP has been injected and the time noted, what are the time intervals for collection of urine specimens?

2. What are some substances in the urine that could interfere slightly with the PSP test?

3. Why is it unlikely that a patient would receive an overdose of PSP dye?

1-2. Clearance and Concentration-Dilution Tests

Clearance tests are designed primarily to measure the efficiency with which the kidney removes certain substances from the blood. The most common clearance tests employed in clinical diagnosis are the urea clearance and creatinine clearance tests. These techniques are used to evaluate kidney function by establishing a relationship between the rate of excretion of a test substance and the concentration of that substance in the blood or plasma. Concentration-dilution tests measure the capacity of the kidney tubules to maintain the specific gravity of urine within physiologically acceptable limits. The normal specific gravity of a random urine specimen is 1.003 to 1.030, and the normal specific gravity for a 24-hour urine specimen is 1.015 to 1.025.

404. Compare the urea clearance and creatinine clearance tests as to specificity, variables, purpose, and degree of accuracy.

Urea Clearance Test. The urea clearance test was the first clearance method to be used clinically and has considerable historical significance. After the urea is filtered by the glomeruli, it is subsequently reabsorbed partially by the tubules; therefore, results of this test are not a true measure of glomerular filtration rate but rather of the overall renal function. Popularity of other clearance tests that measure specific aspects of renal function has increased, and the urea clearance test is now largely of historical interest. Although it is still considered valid by some authorities, a combination of practical difficulties and theoretical considerations have led to its being superseded by more precise tests such as the creatinine clearance and the PAH (p-aminohippuric acid). In the urea clearance test, urea concentration of a diluted urine specimen, as compared to the blood urea concentration, is used as an index of the ability of the kidneys to remove nitrogenous substances from the blood.

Variables. Urea is produced by the liver and is freely filtered through the glomeruli, but is partially absorbed by the tubules. The passive absorption is dependent on (1) the rate of urine flow (ml/min), (2) the amount of urea present, and (3) the state of the tubular epithelium. Variabilities observed in the rate of flow show that, at a low rate of urine flow, up to 60-80 percent of urea may be absorbed and the clearance is therefore low. At a high rate of urine flow, only 30-40 percent of urea is absorbed, and the clearance rate is therefore high. Further, the amount of urea present in the plasma is dependent on the protein content of the diet. A high protein content results in an increased urea level. The urea level can also be influenced by catabolic metabolism resulting from infections or leukemia. Urea clearance values between 60 to 125 percent of normal are considered within the normal range. Values below 70 percent of normal clearance are suggestive of decreased renal function. Elevations of the nonprotein (blood) nitrogenous (NPN) compounds in serum are accompanied by values below 50 percent. Values below 10 percent are suggestive of severe renal impairment.

Since urea clearance is influenced by so many variables, it is a test for neither GFR (glomerular filtration rate) nor tubular absorption and does not warrant continued diagnostic use, since more specific tests are readily available in the clinical laboratory.

Creatinine Clearance. The clearance of creatinine from the blood is primarily an index of glomerular filtration rate. The test is performed in a manner similar to the test for urea clearance, with a few appropriate changes. The time period over which the urine specimens are collected is usually longer for the creatinine test, sometimes covering 24 hours. Further, protein intake of the patient should be controlled in the creatinine clearance.

Creatinine is a normal end product of metabolism with a relatively constant plasma concentration and daily urinary excretion that is not greatly influenced by diet, urine flow rate, or exercise. The reasons for the greater degree of accuracy of creatinine clearances are (1) creatinine is not reabsorbed by the tubules, and (2) fluid intake and excretion affect the clearance of creatinine much less than they affect the clearance of urea.

A suggested procedure for the test is outlined in AFM 160-49. Calculations are performed as follows:

$$C = \frac{UV}{P} \times K$$

where

C = endogenous creatinine clearance,
U = mg/ml urine creatinine,
V = ml urine per minute,
P = mg/ml plasma creatinine, and
K = 1.73 divided by patient's body surface area in square meters.

(Refer to table 1-1.)

Normals range from 75-157 ml per min. This test is sometimes compared with, or substituted for, the inulin clearance test.

Exercises (404):

1. Why is the urea clearance test not a true measurement of glomerular filtration rate?

2. The passive absorption of urea by the tubules is dependent on what three variables?

TABLE 1-1
CORRECTION FACTOR K FOR CREATININE CLEARANCE TEST

Height Cm	Factor (K) to multiply V to obtain corrected V:
175	1.01
170	1.06
165	1.10
160	1.17
155	1.25
150	1.33
145	1.41
140	1.49
135	1.59
130	1.69
125	1.80
120	1.92
115	2.04
110	2.19
105	2.34
100	2.50
98	2.56
96	2.62
94	2.68
92	2.74
90	2.81
88	2.90
86	2.99
84	3.09
82	3.20
80	3.31
78	3.42
76	3.56
74	3.70
72	3.86

Cm = inches X 2.54.

3. What effect would a high protein content of diet have on the urea level?

4. What is the purpose of the creatinine clearance test?

5. Give two reasons why the creatinine test has a greater degree of accuracy than the urea clearance test.

405. Indicate the procedures for the determination of urine concentration by citing physiological factors that affect concentration, techniques, methods of measurement, possible source of error, and normal ranges.

Concentration Tests. Normal kidneys are able to concentrate urine and efficiently remove waste products from the blood. Increased values for the specific gravity of urine are found in febrile conditions, nephritis, and diabetes mellitus. Decreased values occur in diabetes insipidus and chronic nephritis.

Urinary concentration is dependent upon many factors. Three important factors that affect concentration are as follows:

(1) Water deprivation stimulates the release of antidiuretic hormone (ADH) by the pituitary, increasing the water absorption by the distal tubules.

(2) Aldosterone has to do with the reabsorption of sodium by the distal tubules.

(3) The amount of urea excreted by the kidney contributes to significant changes in the values of the specific gravity and osmolality. The urine concentration is best measured by the refractometer or by obtaining the osmolality by freezing point determination.

Methods of measurement. Urine concentration is measured by three methods: (1) specific gravity, (2) total solids or indirect estimation of specific gravity by refractometry (refractive index), and (3) osmolality. The specific gravity of urine is usually determined by a hydrometer commonly called a urinometer. A light refractometer is used for refractive index, which is usually calibrated in terms of total solids or specific gravity. The principle of freezing point depression is employed by osmometers for measuring osmolality. An osmometer is an instrument that determines the concentration of free particles in a solution, the osmolality, by measuring the freezing point. The lower the freezing point, the higher the osmotic pressure. The instrument is calibrated in milliosmols and requires a 2-ml sample.

Fishberg concentration test. This test determines the ability of the kidney to vary the concentration and volume of urine according to the food and fluid intake. The patient eats a high protein evening meal with fluid intake restricted to not more than 200 ml. No further intake of food or liquid is allowed until the test is completed. The patient is allowed to void and discard urine during the night, but is instructed to collect specimens at 0600, 0700, and 0800 hours. Measure the volume and the specific gravity or osmolality of each specimen.

If the patient ingests more fluid than allowed, the test will not be valid. Normally, the specific gravity of at least one of the specimens will be 1.022-1.032, and the osmolality will be from 800-1300 mOsm/liter. A well-functioning kidney will produce up to 300 ml of urine with a specific gravity of 1.022 or higher. With increased kidney impairment the specific gravity approaches 1.010.

Dilution tests. The ability of the kidney to dilute urine is sometimes evaluated by means of dilution tests. Urine dilution tests are also a measure of renal tubular function but are infrequently performed. Dilution tests are generally less sensitive than concentration tests as indicators of impaired renal function, but the two tests are independently influenced by several renal and extra-renal conditions.

Although the test is contraindicated in cases of cardiac or renal edema, it may be of value in conditions such as adrenal insufficiency. Individuals normally excrete 1200 ml (or slightly more) of urine per day. A patient with adrenal insufficiency (Addison's disease) usually excretes 800 ml of urine or less in a day.

One procedure for conducting a dilution test is as follows: The patient does not eat or drink after 2000 hours. At 0800 hours he empties his bladder and drinks 1500 ml of water within 45 minutes. Eight separate specimens are collected at 30-minute intervals thereafter. The specific gravity should be 1.003 or less in at least one specimen, and the total volume of urine voided should be over 1200 ml. The osmolality of at least one sample should fall below 80 mOsm/liter and may decrease to 37 mOsm/liter.

Exercises (405):

1. Concentration tests are dependent upon what three given factors?

2. What two techniques are considered best in measuring urine concentration?

3. On what principle does the osmometer work and what is the unit of measure?

4. What is a possible source of error that could invalidate the Fishberg concentration test?

5. What is the normal range of the osmolality for the Fishberg concentration test? Volume? Specific gravity?

6. When the dilution test is compared with the concentration test, which is considered the least sensitive as an indicator of impaired renal function?

7. What is the normal range of osmolality for the dilution test?

Gastric Analysis

THE ANALYSIS of gastric secretion has remained an important role in clinical diagnosis and evaluation of therapy. As with many other laboratory tests, information derived from gastric analysis is by itself rarely of diagnostic significance, but must be interpreted in light of the patient's history and with results of other relevant clinical, X-ray, and laboratory tests. In this chapter you will study the basic anatomy and physiology of the stomach and laboratory procedures in gastric analysis.

2-1. Basic Anatomy and Physiology of the Stomach

Most foods are not naturally in a form which can be absorbed directly into the blood. The stomach serves as a storage area for food until it can be broken down in the intestine. While the food is in the stomach, a mixing action and certain digestive juices reduce the food to a semiliquid called chyme. The most active components of the digestive juices are hydrochloric acid, lipase, pepsin, and, in infants, rennin.

406. Identify the terms that describe the general features of the stomach.

General Features of the Stomach. The stomach may be described as an expanded part of the digestive tract between the esophagus and the small intestine. The esophagus enters the stomach on the right side just below the fundus. A band of visceral muscle at the bottom of the esophagus constitutes the cardiac sphincter, which remains closed except when a peristaltic wave, or a counterforce such as nausea, causes it to open. Food is forced into the stomach by peristalsis independent of the force of gravity. The narrowing portion of the stomach, below a curving main portion called the body of the stomach, is the pyloric portion. The pylorus is separated from the small intestine by a pyloric sphincter. Peristaltic movements, which begin near the middle of the stomach, cause food to move into the intestine. Control of the pyloric sphincter is rather complicated and precise. The area between the pylorus and the body of the stomach constitutes the pyloric antrum and canal, which are labeled in figure 2-1. When it is empty, the stomach is arranged in anatomical folds termed "rugae." A distended stomach has a capacity of 1 to 1 1/2 quarts in an adult of average size.

Histologically, the stomach has certain general microscopic features. There are millions of simple tubular glands. Glands in the esophageal area of the stomach differ somewhat from those in other areas of the stomach. Gastric glands are made up of three kinds of cells: the chief cells which secrete enzymes, the parietal cells which produce hydrochloric acid, and the mucous cells. The gastric juice wells up through openings in the mucosa, which are referred to as gastric pits. These glands extend to a zone histologically identified as the lamina propria, below which lies the muscularic mocosae.

The surface of the stomach consists of simple columnar epithelium, which extends down to line the pits. This epithelium protects the surface of the stomach. Normal and viable stomach tissue is not affected by the digestive juices present in the stomach, although immediately after death, cells of the stomach undergo autolysis. As might be expected, ulceration of the mucous membrane sometimes occurs. Contrary to what you might expect, wounds of the stomach heal perfectly well even in the absence of the protective columnar epithelium. Psychogenic factors may complicate healing processes.

Exercises (406):

Match each term in column B with the correct definition in column A by placing the letter of the column B item beside the number of the column A item that most nearly describes it. Each element in column B may be used once.

Column A

____ 1. Dome at top of the stomach.
____ 2. Rhythmic action of stomach.
____ 3. Openings in gastric mucosa.
____ 4. Valve into small intestine.
____ 5. Enzymatic self-destruction.
____ 6. Folds in the stomach.

Column B

a. Peristalsis.
b. Gastric pits.
c. Fundus.
d. Lamina propria.
e. Pyloric sphincter.
f. Parietal cells.
g. Columnar epithelium.
h. Autolysis.
i. Chief cells.
j. Rugae.

Column A

___ 7. Cells which secrete HC1.
___ 8. Tissue beneath gastric glands.
___ 9. Column-shaped epithelial cells which line the stomach.
___ 10. Cells of the stomach which secrete an enzyme.

407. Tell how hydrochloric acid is formed in the stomach, cite the average pH of gastric juice, and identify terms related to gastric digestion.

Gastric Digestion. The primary functions of the stomach are (1) storage of food, (2) digestion, and (3) bactericidal effect. All are important, but not vital, since complete removal of the stomach (total gastrectomy) can be tolerated. Food which passes through the cardiac orifice of the stomach accumulates in the lower curved portion. Further accumulation of food causes some stretching of the stomach walls. Peristalsis of the stomach is regulated according to the amount of food present. Secretion of gastric juices is caused by nerve impulses which are conducted from the brain through the vagi nerves to the glands of the stomach. These impulses are the result of sensory or psychic stimuli, including the sight or smell of food. Stretching the stomach walls, as well as the chemical nature of certain foods, also influences gastric secretion. Alcohol and histamine are both used in the hospital to stimulate gastric secretion.

Gastric juice consists of 98 percent water; the rest includes pepsin, hydrochloric acid, inorganic salts, mucin, and the so-called intrinsic factor. This is a factor present in gastric juice which is essential for the prevention of anemia. Its exact nature is obscure, but it is thought to be a mucoprotein secreted in the fundus and cardiac portions of the stomach. Gastric juice has a pH of 0.9 to 1.5, and the HCl secreted by the

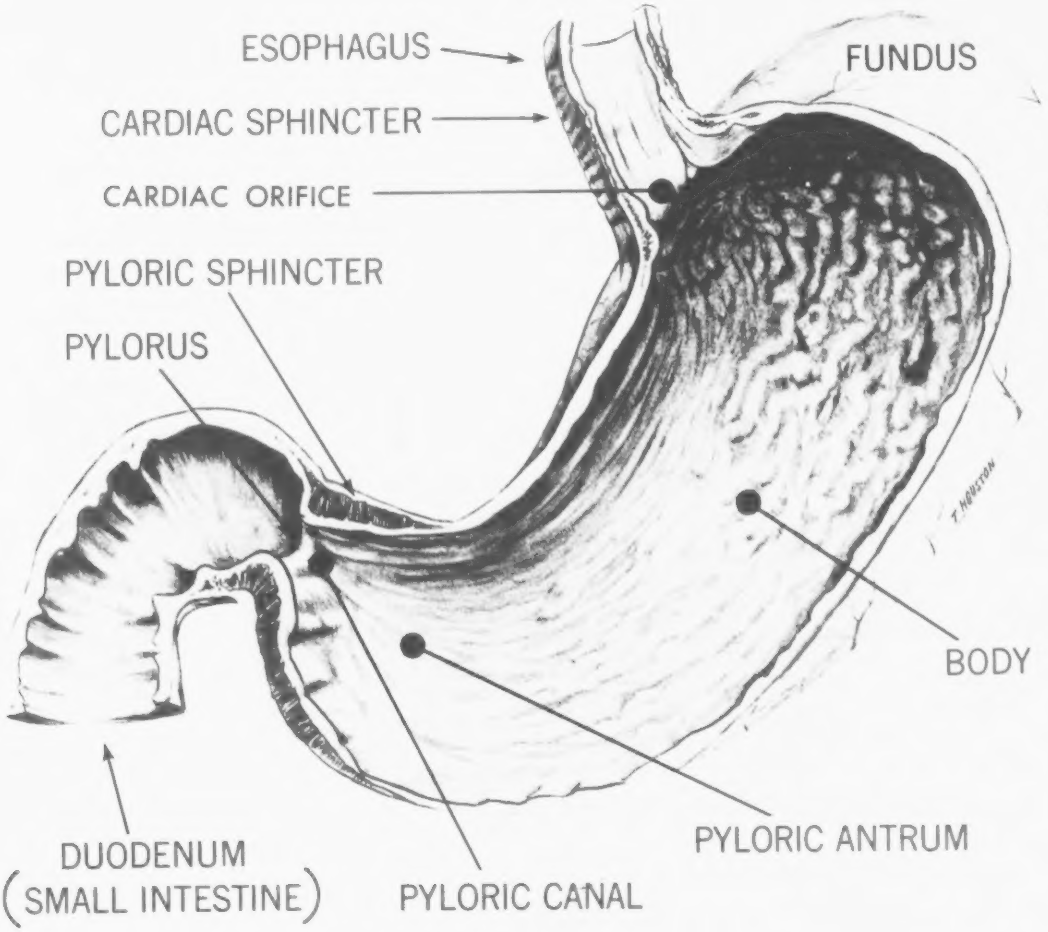


Figure 2-1. The stomach.

parietal cells has a concentration of 0.15N. The amount of gastric juice found in the fasting stomach is usually about 50 ml, with approximately 2500 ml secreted per day by an adult. Patients with a duodenal ulcer secrete twice that amount. The actual process of HCl formation in a parietal cell is essentially that of the chloride shift described in an earlier chapter. Carbonic acid, H_2CO_3, is the source of the hydrogen that combines with chloride ions which diffuse into the cell from the surrounding plasma.

Constituents of Gastric Juice. Besides the constituents of gastric juice mentioned, certain other substances are present. They include pepsin, rennin, and lipase.Together with the action of the acid, pepsin breaks proteins into proteoses and peptones. Rennin, which is of little consequence to adults, converts milk protein (casein) to paracasein, and lipase acts on fats to produce fatty acids and alcohols. The lipolytic action of gastric juice is not considered very important, because of the greater activity of pancreatic lipase in the intestine. Gastric lipase is responsible for the release from butter fat of butyric fat, a product which causes the characteristic disagreeable odor of gastric contents. Unlike the intestine, relatively little absorption takes place in the stomach. Water and alcohol are examples of substances which can be absorbed directly into the blood from the stomach.

Exercises (407):

1. Explain how HCl is formed in the stomach.

2. What is the average pH of gastric juice?

In questions 3-8, match the terms in column B with the definitions to which they are closely related in column A by placing the letter of the column B item beside the number of the column A item. Each column B item may be used once.

Column A

___ 3. Enzyme which acts on proteoses.
___ 4. Nerves to the stomach.
___ 5. Enzyme which acts on fats.
___ 6. An organic acid which is the source of hydrogen for HC1.
___ 7. Surgical removal of the stomach.
___ 8. Used to stimulate gastric secretion.

Column B

a. Vagi.
b. Lipase.
c. Gastrectomy.
d. Carbonic acid.
e. Pepsin.
f. Alcohol and histamine.

408. Briefly state basic principles and terms associated with the measurement of gastric acidity.

Units of Measurement. Older literature expressed gastric acidity as "degrees"; one degree represented each milliter of 0.1N NaOH needed to titrate 100 ml of gastric juice to the desired end point. The two end points used were (pH 2.8-3.5) the end point for Topfer's reagent for "free acid," and the pH 8.2 range obtained with phenolphthalein for "total acid."

At pH levels below 3.5 all the HCl was thought to exist as dissociated or free acid, while above that point the hydrogen ion was though to be buffered by organic acids and peptides. The combined acid included HCl combined with protein, acid phosphatase, and organic acids. Thus, the total gastric acidity depended upon the presence of both free and combined acidity.

Older concepts of gastric acidity were supported by titration curves obtained from the neutralization of gastric acid with sodium hydroxide. These curves resembled titration curves of an aqueous solution of hydrochloric acid at pH values below about 2.8, but above this pH the curves more closely resembled those of a buffer mixture compound of a weak acid and its salt. Authorities indicated that such curves failed to take into account the buffering effect of the various test meals then in use as gastric stimulants, which could considerably affect the outcome of the test.

It has been recommended that the older terms of "free," "combined," and "total" acid be avoided. The concept of free and combined as parts of the total acid has been shown to have neither physiologic nor physicochemical validity. Gastric secretions can be more meaningfully described in terms of three measurements performed on each sample of gastric secretion. The three measurements are:

(1) Volume. Expressed in milliliters.

(2) Titratable acidity. Expressed in milliequivalents per liter. A suitable aliquot of gastric secretion is titrated with 0.1N NaOH to neutrality (pH of 7.0 or 7.4). The end point should be measured electrometrically with a suitable pH meter. If a pH meter is not available, the end point may be determined colorimetrically with phenol red indicator, which changes color from yellow to red within a pH range of 6.8 to 8.4.

(3) pH. Measured electrometrically.

Acid Output. The acid output is expressed in milliequivalents per liter. The value for each sample is calculated by multiplying its volume in milliliters by the titratable acidity and dividing by 1000. Along with the measured volume, titratable acidity, pH, and the calculated acid output for each individual sample, you will usually report the total volume and total acid output for a given test. The total volume and total acid output are found by adding the individual sample values.

Basal acid output. In the study of basal secretion, a 1-hour collection consisting of four 15-minute samples is made. The basal acid output is in milliequivalents per hour and is reported as the sum of the acid outputs for the four samples.

Maximal acid output. In the augmented or maximal histamine test, the quantity of acid secreted in the hour following the injection of histamine is expressed in milliequivalents and reported as the maximal acid output. Maximum secretion is measured after subcutaneous administration of an augmented dose of 0.05 mg/kg histamine dihydrochloride of 2 mg/kg Histalog. After the injection, the gastric secretion is collected by continuous aspiration for 60 minutes in four 15-minute collections.

Peak acid output. The greatest acid output in two successive 15-minute periods in the augmented histamine test is referred to as the peak acid output.

Diagnostic Findings. There is a correlation between the gastric secretory activity and certain clinical conditions. Terms used to describe the result of gastric secretion tests are explained in the following paragraphs.

Anacidity. Anacidity was formerly defined as the absence of free acid, usually taken to mean a failure of the gastric secretory pH to fall below 3.5. Most authorities now define anacidity as a failure of the pH to fall below either 6.0 or 7.0 in the augmented histamine or Histalog tests. In the augmented histamine test, anacidity is found in adults with pernicious anemia or gastric carcinoma. Anacidity nearly always accompanies pernicious anemia. It has been implicated in a variety of conditions, such as hypochromic anemia, rheumatoid arthritis, steatorrhea, aplastic anemia, myxedema, and nutritional megaloblastic anemia. It is often found in the asymptomatic relatives of patients with pernicious anemia. Some authorities use "achlorhydria" synonymously with anacidity, but the term is defined differently by others. Some investigators have defined "achlorhydria" and "hypochlorhydria" with such fine lines of distinction that the terms have little clinical usefulness. It has been recommended that these terms by avoided.

Zollinger-Ellison syndrome. In this condition, gastric-secreting pancreatic tumors bombard the parietal cells with continuous high-level stimulation of high acid output. A basal acid output above 15 mEq per hour is suggestive of Zollinger-Ellison syndrome. The mean basal acid output ranges from 4 to 5 mEq per hour. Lower values occur in females and in the aging. The range for maximal acid output in normal males has been found by researchers to be 4.9 to 38.9 mEq per hour. In addition to marked hypersecretion, patients with the Zollinger-Ellison syndrome have a high ratio of basal to maximal acid output.

Stimulating gastric secretion is almost exclusively accomplished by the medical and nursing staff of Air Force facilities, as is the use of a gastric tube for withdrawal of stomach contents. It is not necessary, therefore, that you, the laboratory technician, be familiar with the details of these procedures. You should be aware of the nature of gastric stimulants from the viewpoint of physiological chemistry, however. Although interpretation of the response is not a laboratory problem, you should appreciate that there are now three measurements to be performed as accurately as possible: volume, titratable acidity, and the pH.

Exercises (408):

1. Why should the terms "free," "combined," and "total" acidity be avoided in the study of gastric secretion?

2. What are the three terms now used in the measurement of gastric secretion?

3. How is the acid output expressed and calculated?

4. Basically, how is the study for basal secretion done?

5. What is the maximal acid output?

6. What does "anacidity" mean?

7. What is the Zollinger-Ellison syndrome?

8. Why should the terms "achlorihydria" and "hypochlorhydria" be avoided?

2-2. Laboratory Procedures in Gastric Analysis

The acidity of gastric fluid is of primary concern to the clinical laboratory because of its relationship to clinical conditions. There are two laboratory methods for gastric analysis: (1) the intubation technique, which allows measurement of both basal and maximal (histamine-stimulated) secretion, and (2) the tubeless technique which investigates the ability of the stomach to produce acid. These methods will be discussed in this section.

409. Indicate whether given statements correctly reflect the normal and abnormal physical characteristics of gastric fluid.

Physical Characteristics of Gastric Fluid. The following characteristics should be noted as you assay gastric fluids. Any deviation from the normal should be reported.

Color. A normal fasting gastric specimen is opaque to clear, with a slight gray to pale yellow color.

Presence of mucus. Gastric fluid may be difficult to pipette because of its viscous nature. A 10-ml serological pipette, equipped with a wide-bore tip and a bulb, works well in transferring the specimen. A specimen may also be carefully measured in a 10-ml graduated cylinder. Preliminary filtration through gauze is helpful. Abnormal amounts of mucus may indicate gastritis, with inflammation of mucous tissue. Excessive mucus may cause falsely decreased acid values.

Volume. The volume of the gastric specimen should be measured.

Odor. This is usually less important than other features, though an unusual odor may be of interest. For example, a fecal odor is typical of intestinal obstruction.

Particulate matter. Food particles are not normally present in a fasting specimen unless there is a problem of stasis.

Presence of blood. Blood which has accumulated in the stomach is brown, usually described as appearing like coffee grounds. Bright red blood due to fresh bleeding is less common unless it results from trauma or injury. The presence or absence of blood is extremely important and should be tested for by the guaiac or benzidine test if suspected.

Exercises (409):

You are performing a gross examination gastric fluid to determine by its physical characteristics whether it appears normal or abnormal. Indicate whether each of the following statements concerning the physical assay of gastric fluids is true (T) or false (F) and correct those that are false.

T F 1. Food particles present in a fasting specimen indicate a problem of stasis.

T F 2. Preliminary filtration of the specimen through gauze will be of little value.

T F 3. A slight to moderate yellow-green color of the specimen could be considered normal.

T F 4. The volume of each specimen should be measured.

T F 5. Contents with a brown color and the appearance of coffee grounds are an indication of food particles in the fluid.

T F 6. Excessive mucus may cause falsely decreased acid values.

410. Describe the intubation and tubeless methods for determination of gastric acid secretion in terms of purpose, reagents, procedures, and interpretation.

Intubation Technique. In the determination of gastric acid secretion, gastric fluid is collected either under basal physiologic conditions or following stimulation with a pharmacologic stimulus, such as histamine or Histalog. The titratable acidity of each specimen is determined by titration to pH 7.0 with sodium hydroxide, and the acid output is calculated. The reagent used for titration is 0.1N NaOH. A simple version of the titration procedure is outlined below.

Procedure. First let's look at the step-by-step method of performing the titration.

(1) Measure the volume of each specimen and determine the pH with a suitable pH meter.

(2) Titrate a 5-ml aliquot of each specimen to pH 7.0 with 0.1N NaOH.

Calculation:

$$\text{Titratable acidity (mEq/liter)} = \text{ml of NaOH} \times 20$$

$$\text{Acid output (mEq H}^{+}\text{)} = \frac{\text{titratable acidity} \times \text{specimen volume in ml}}{1000}$$

The pH should be measured with a reliable pH meter that can be read to 0.001 pH unit. Two calibrating buffers are required, one with a pH of 7.0 and one with a pH of 1.68. The pH 7.0 buffer may be obtained commercially, and the pH 1.68 buffer can be prepared from potassium tetraoxalate obtained from the National Bureau of Standards. Dissolve 12.61 gm of this reagent in distilled water and dilute to exactly 1 liter. This solution should have a pH of 1.675 at 20° C, 1.679 at 25° C, and 1.683 at 30° C.

If a suitable pH meter is not available for monitoring the titration, titratable acidity may be determined using phenol red indicator, 0.1 percent aqueous. (Dissolve 0.1 gm acid in 5.7 ml 0.05N NaOH and dilute to 100 ml with water.) Add 1 to 2 drops of indicator to the aliquot of gastric secretion. Continue titration until the color of the indicator matches that of a suitable control buffer at pH 7.0. If the specimen of gastric secretion is of insufficient volume for titration, add water and make the correct dilution factor in the calculation for titratable acidity.

Interpretation. The rate of secretion in milliequivalents per hour may be calculated by dividing the total amount secreted by the collection time in hours (mEq/hr). Normal values are as follows:

Fasting residual volume	20-100 ml
pH	< 2.0
Basal secretion test	0-10 mEq/hour
Augmented histamine test	1-40 mEq/hour

Tubeless Gastric Analysis. An ion exchange resin is available commercially (Diagnex Blue, Squibb) which makes use of a dye, azure A, as an indicator. The patient is given a packet of the resin granules and instructed to swallow them without chewing, as outlined on each package. The amount of dye that appears in the urine depends upon displacement from the resin by HCl in the stomach. A color chart is used to estimate the amount of dye in the urine, which is an index of the amount of HCl in the stomach.

This test is considered valuable as a screening test only. It is preferred to passing a gastric tube only because it is simple and does not cause the patient discomfort. Its reliability, however, is not beyond question.

False positive results may be caused by the following:

a. Presence of cations in the gastric contents that have strong affinity for the resin and are capable of displacing azure A; for example, aluminum, magnesium, barium, calcium, iron, or kaolin.

b. Gastric hypermotility with rapid emptying.

False negative results may be caused by:

a. Insufficient stimulation of gastric secretion by caffeine sodium benzoate.

b. Pyloric obstructions and malabsorption syndromes that interfere with intestinal absorption of the liberated azure A cations.

Within the past few years there have been attempts to take a new approach to the subject of gastric analysis in keeping with up-to-date methods and equipment. Some investigators have proposed the determination of hydrogen ion concentration from electrode pH measurements. In theory, this is the most accurate method. It requires precise measurements of pH with a glass electrode and determination of the sum of potassium and sodium. This more rigorous physicochemical approach does not appear to be justified for routine clinical use.

Exercises (410):

1. What is the reagent used for titration of the aliquot of gastric secretion?

2. What is the first step before determination of the titratable acidity?

3. What should the pH of the two buffers read?

4. If a suitable pH meter is not available, how may the titratable acidity be determined?

5. What should you do if the sample for titration is inadequate?

6. How is the rate of secretion computed and expressed?

7. How is a tubeless gastric analysis accomplished?

8. Of what value is a tubeless gastric analysis?

Special Chemistry Tests

SPECIAL CHEMISTRY tests are "special" only because these tests are usually performed only in large clinical laboratories. This does not mean they are unnecessary at small medical facilities, but special chemical analyses usually require unique, costly equipment and technicians trained in the special techniques. Because of the cost and complexities of these tests, it is important for all technicians at reference and referral laboratories to be aware of general technical considerations involved with each "special" test.

3-1. Referral Chemistries

In this section we will consider various referral type analyses. The discussion of electrophoresis develops from variations in technique. Chromatography is reviewed in principle and in its general application. The physiology and quantitation of serum iron is developed in the light of its clinical importance, while recent dramatic innovations in protein-bound iodine (PBI) analysis are described in comparison to standard manual methods.

These are the more common special chemistry tests. You will need to know this information to understand your responsibilities in collection, preservation, and shipment of specimens. You may also require the technical knowledge of special chemistry procedures to actually perform some of these tests in a large reference laboratory.

411. Identify the procedures for electrophoresis in terms of definition, principle, use, methods, and relative advantages of cellulose acetate strips.

Electrophoresis. The migration of charged particles suspended in an electrolyte solution under the influence of an electric current flow is known as electrophoresis. Colloidal particles, such as protein, although invisible to the unaided eye, are actually in suspension rather than in true solution. Some of the particles (fractions) which may be separated by electrophoresis because of their different mobilities include proteins, lipoproteins, hemoglobins, amino acids, and many other substances of medical and biological importance. The widespread application of electrophoresis is quite recent. However, the concept of electrophoresis is not new.

Principle. In 1861, a German investigator, Quincke, demonstrated a relationship between the speed of particle migration and the electric potential gradient, as well as the relationship between speed of migration and pH of the suspending medium. Egg albumin was one of the proteins first investigated in detail. The activity of albumin in an electrical field was studied by W.B. Hardy in the latter part of the 19th century. Writing in the *Journal of Physiology* in 1899, Hardy reported that protein particles have ". . . this very interesting property, that their electrical characters are conferred upon them by the nature of the reaction, acid or alkaline . . . ." In other words, particles such as proteins, suspended in an aqueous medium, will have either a positive or a negative charge, depending upon the pH of the solvent and the nature (size, charge, etc.) of the particles. The pH at which a protein is electrically neutral is the point at which it will not migrate in an electrophoretic system. This particular pH, characteristic for each protein, is referred to as its *isoelectric point.* The major protein fractions of human serum are in the anionic form at an alkaline pH and hence will migrate to the anode. They are usually separated at a pH of 8.6.

Quantitative separation of proteins. A major problem in the development of electrophoresis has been quantitating each fraction which can be distinguished. Keep in mind that both *rate* and *direction* of movement in a field depend upon the nature of the particle itself as well as the pH of the solution. Albumin, for example, can be separated from globulin because it migrates at a faster rate due to the size and charge of the albumin molecule. Quantitating the albumin is quite another matter, however. The first practical means of quantitatively separating proteins was described in 1937 by Nobel Prize winner Arne Tiselius of Sweden. The classical Tiselius technique was quite expensive and time consuming. The Tiselius apparatus used *moving boundary electrophoresis.* Today it is used in research and special studies, but is not particularly useful in a hospital laboratory.

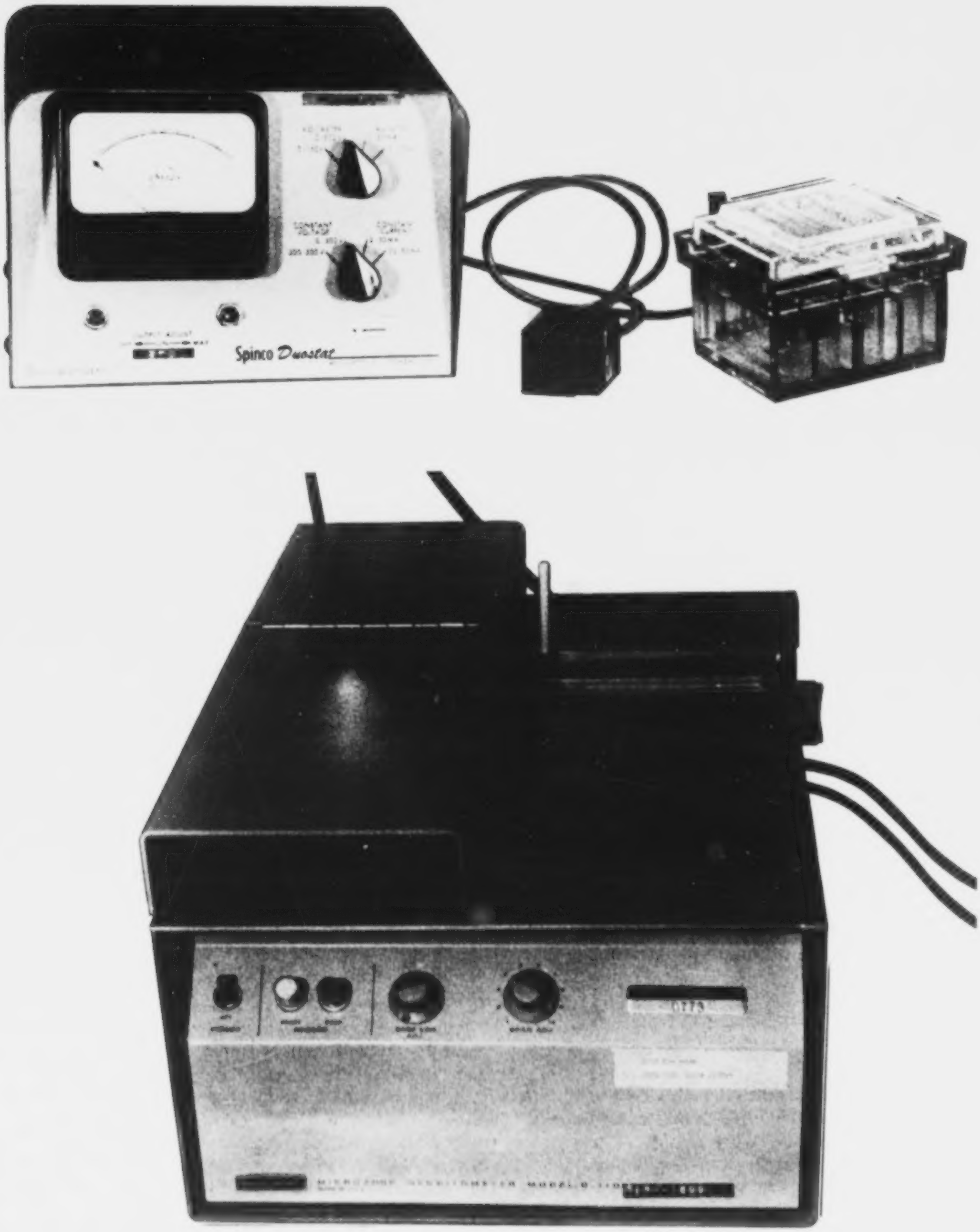

Spinco Duostat

Figure 3-1. Equipment to perform zone electrophoresis using cellulose acetate strips.

Methods. Simpler methods of electrophoresis have now been developed. The use of supporting media to prevent mixing of the fractions by convection is a more recent method of quantitatively separating the fractions and is more practical for clinical use. These methods generally employ a solid matrix to eliminate the problems caused by convection currents during electrophoresis and to stabilize the separation boundaries. Filter paper was the first such matrix explored. The development of other supporting matrices like cellulose acetate, agar gel, starch gel, and acrylamide gel, and the improvement in electrophoretic equipment have resulted in very sophisticated electrophoretic techniques. The use of such supporting media through which the proteins migrate is referred to as *zone electrophoresis.*

Advantage of cellulose acetate strips. The use of cellulose acetate strips as a support medium has gained wider clinical application as compared to paper strips. Speed, improved resolution, and microsample size are distinct advantages with this supporting medium. Resolution of 0.25 microliters of serum is completed in half an hour as opposed to 16 hours on paper. (The terms microliter (μ1) and lambda (λ) are synonymous with and equal to 1/1000 ml). In addition, eight different specimens can be placed on one strip of cellulose. The disadvantages of paper as a support medium—irregular structure, variability of quality, and "tailing" of protein fractions (failure of proteins to migrate as a compact fraction with subsequent blurring of the pattern and staining of the zones between the fractions)—are largely overcome by using cellulose acetate strips. Familiar to many clinical laboratories is the equipment pictured in figure 3-1, which uses cellulose acetate strips.

Cellulose acetate has the following advantages over paper:

a. Cellulose acetate strips can be used without any special preparation.
b. Wet cellulose acetate is stronger than any other supporting medium and thus easy to handle.
c. Very small quantities of sample can be conveniently applied and are sufficient.
d. Only a short time is required for electrophoresis.
e. Better resolution of bands of components is achieved.
f. Cellulose acetate strips can be easily cleared after staining.
g. Good transparency of the strips facilitates quantitation by densitometry.

One of the simplest and most convenient electrophoretic systems for routine laboratory use is the Beckman Microzone cellulose apparatus. For those laboratories that have previously used the paper strip method from the same source, the Analytrol scanner can be quickly adapted by an attachment for scanning and integration of the tiny stained patterns produced by the Microzone method.

Exercises (411):

Match the following by placing the letter of the column B item beside the number of the column A item which most nearly describes it. Each element in column B may be used once, more than once, or not at all.

Column A

____ 1. Migration of charged particles in an electrolytic solution resulting from an electric flow of current.
____ 2. Nature of particle (size, charge) and pH of the medium.
____ 3. pH at which a protein fraction is electrically neutral and does not migrate.
____ 4. First type of matrix used as support medium in electrophoresis.
____ 5. Irregular structure, variability of quality, and "tailing" of protein fractions.
____ 6. Starch gel, agar gel, cellulose acetate, and acrylamide gel.
____ 7. Use of supporting media through which proteins migrate.
____ 8. Can be used without special preparation, is stronger than other supporting media, and can be conveniently used in very small quantities.
____ 9. Failure of proteins to migrate as a compact fraction with subsequent blurring of zones between fractions.
____ 10. Apparatus that can be adapted, by an attachment, for scanning and integration of the tiny stained patterns produced by the Microzone method.

Column B

a. Analytrol scanner.
b. Disadvantages of filter paper strips.
c. Supporting matrices used in zone electrophoresis.
d. Electrophoresis.
e. Tailing.
f. Factors that determine the rate and direction of migration of a particle in electrophoresis.
g. Advantages of cellulose acetate strips.
h. Isoelectric point.
i. Filter paper.
j. Zone electrophoresis.

412. Indicate whether given statements correctly reflect the procedures for processing and reading cellulose acetate strips, use of electrophoresis studies, and methods of processing and differentiation of hemoglobin electrophoresis.

Procedure With Cellulose Acetate Strips. After visual inspection of the strip, impregnate it with buffer by carefully floating it on the surface of the buffer

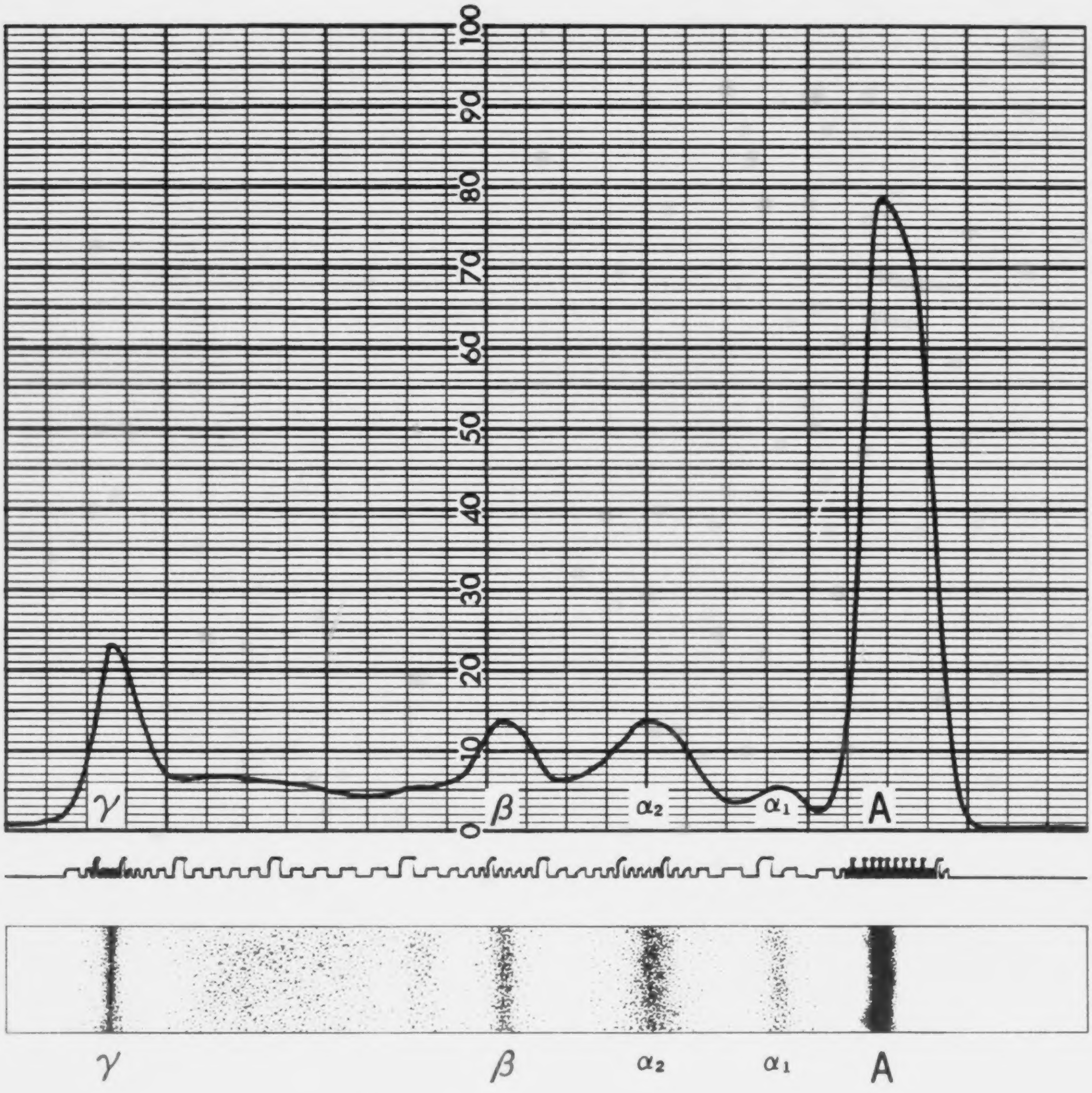


Figure 3-2. Cellulose acetate electrophoretic pattern of serum from multiple myeloma.

solution and allowing the strip to absorb the buffer from underneath. Then immerse the strip completely in the buffer. After immersion, lift the acetate strip out and blot it between filter papers to remove excess buffer, but keep it moist. After blotting, mount the membrane on the bridge without delay and position it in the cell, which has previously been filled with buffer. From this point, remove the cell cover only briefly during the application of serum so that the membrane does not dry out. The amount of serum applied is very small—about 0.25 microliter. Include a control serum with every batch. The running time is 20 minutes or the time specified in the manufacturer's instructions. Carry out the staining and washing steps as soon as the membrane is removed from the tank. Reference dyes are often used to monitor the rate of migration. For example, albumin with bromphenol blue is frequently used as an internal control in electrophoresis. After the membranes have been washed, rinsed, cleared, and excess moisture removed, place them in an oven until dry. After they have been removed from the oven, quantitation is done with the Microzone scanning attachment.

Quantitation. Reading from anode to cathode on the strip, serum fractions are albumin, alpha 1, alpha 2, beta, and gamma (α_1, α_2, β and γ) globulin. The relative amount of each component may be charted with a scanning device which measures the density of

TABLE 3-1
NORMAL VALUES FOR SERUM PROTEIN FRACTIONS BY CELLULOSE ACETATE ELECTROPHORESIS

Component	*Percent Total Protein*	*g./100 ml*
Total Protein		6.5 to 8.2
Albumin	54 to 74	3.7 to 5.7
Globulins		
Alpha 1	2.1 to 4.2	0.1 to 0.3
Alpha 2	4.6 to 13.0	0.4 to 1.0
Beta	7.3 to 13.5	0.5 to 1.0
Gamma	8.1 to 19.9	0.5 to 1.5

each band by a photocell, as indicated in figures 3-2 and 3-3. Chart a curve with peaks to represent each fraction, which may then be expressed quantitatively as a percent of the total. Determine percent from the integration units marked on the axis by the scanning instrument. To determine the actual amount of component in grams percent or milligrams percent, multiply the percent figure by a total concentration value obtained by some conventional means of analysis, such as the biuret method for proteins. If a scanning instrument is not available, bands may be eluted from segments of the paper strip with a suitable solvent and read in a cuvette with an ordinary spectrophotometer. Normal values for protein fractions in serum are given in table 3-1. Serum specimens are stable for at least 3 days at room temperature and for at least a month if refrigerated.

Use. Electrophoretic studies are clinically useful in patients with liver diseases, myeloma, chronic infections, and sickle cell anemia, as well as in certain other conditions. Compare the tracing of serum from multiple myeloma in figure 3-2 with the normal serum protein electrophoresis in figure 3-3.

Hemoglobin Electrophoresis. All hemoglobins move from the area of the cathode (–) application point to the area of the anode (+) in the following progression: (1) Closest to the application point, which may be faintly visible, where there is an equally faint nonhemoglobin band of CA (carbonic anhydrase and/or catalase); (2) then toward the anode, in order of increasing speed of migration, A_2 (faint) = C and E < S = D < F = G < A < A_3 (faint) < H = Bart's (in newborns). The equal sign implies that by the method described (cellulose acetate), these hemoglobins have identical migration speeds and cannot be differentiated. For example, HbA_2 cannot be separated from HbC and HbE, and HbS cannot be separated from HbD, but they can be differentiated by other methods, such as the sickling test, alkali denaturation test, and citrate agar electrophoresis.

Differentiation of hemoglobins. Regardless of the electrophoretic method used, different types of hemoglobin may have the same mobility, which will require additional chemical and physical methods of differentiation. The unknown hemoglobin is identified by comparing its speed of migration with that of a known control hemoglobin. The control hemoglobin is examined first; all unknown hemoglobins that have the same speed as control HbA, for example, are identified as HbA.

Citrate agar gel electrophoresis is used to separate HbS from HbD and HbE from HbC, which cannot be separated with cellulose acetate.

Starch block electrophoresis may be used as the supporting medium in diagnosis of thalassemia minor. This has a distinct advantage in the separation of HbA and A_2 fractions necessary for the diagnosis of this disease. It has the disadvantage, in addition, of being very difficult to use; permanent records can be obtained only with photographs.

Medium. Cellulose acetate is the medium of choice in many laboratories for both routine serum and hemoglobin electrophoresis. The advantages in serum protein fractionation have already been mentioned. These same factors—resolution, speed, and microsample size—are also desirable in hemoglobin electrophoresis. Cellulose acetate electrophoresis will resolve (separate) HbA_2 from HbS and HbA from HbF in less than 2 hours of electrophoresis. HbF concentrations of less than 2 percent have been quantitated on cellulose acetate strips. In addition, the strip may be cleared to transparency for more accurate quantitation of fractions. These advantages and the marketing of modification kits for existing paper electrophoresis equipment have enhanced the use of this medium for routine electrophoresis in the clinical laboratory.

Preparing specimens. Various techniques are used in preparing blood specimens for separation of hemoglobin variants. In one method of preparing a hemolysate, red cells are washed with saline, after which a volume of water equal to the original volume and 0.4 volumes of touene are added. Centrifugation separates cellular debris, resulting in a clear hemolysate containing the hemoglobin. The use of a buffer is sometimes advocated to avoid possible loss of certain abnormal hemoglobin fractions. Another method of preparing a hemolysate is by freezing and thawing, though some investigators find this produces some protein denaturation. The maintenance of known abnormal control hemolysates is desirable. Clear hemolysates may be stored for months in sealed capillary tubes at –10° C. Capillary tubes provide sufficient small quantities and reduce the risk of excessive denaturation from repeated thawing and freezing of single large specimens. Denaturation of

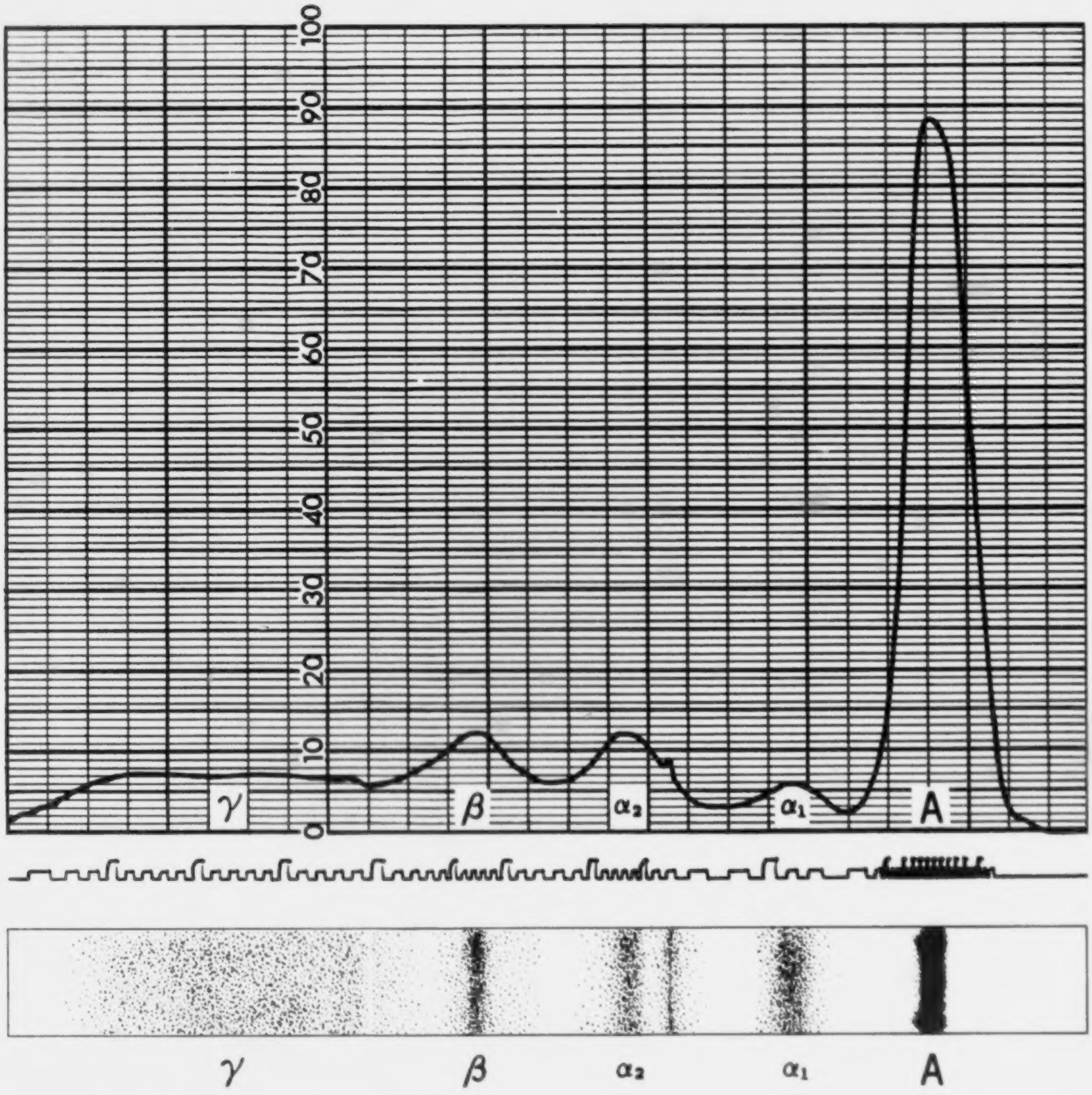


Figure 3-3. Cellulose acetate electrophoretic pattern of normal human serum. A, albumin; α_1 and α_2, alpha globulins; β, beta globulins; and γ, gamma globulins.

control samples will be evidenced by increased trailing (spreading) of the protein bands.

Exercises (412):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

T F 1. The cellulose acetate strip is impregnated by carefully floating it on the surface of the buffer solution and allowing the strip to absorb the buffer from underneath.

T F 2. After immersion of the acetate strip, it is lifted out and blotted between filter papers to remove all moisture.

T F 3. A control must be included with each batch.

T F 4. Reference dyes are often used to monitor rate migration.

T F 5. Reading from anode to cathode on the strip are albumin, alpha 1, alpha 2, gamma (α_1, α_2, and γ) globulin, and beta globulin.

T F 6. Serum specimens are stable for 5 days at room temperature, and for at least a month if frozen.

T F 7. Electrophoretic studies are clinically useful in patients with liver diseases, myeloma, chronic infections, and sickle cell anemia.

T F 8. Hemoglobin A_2 can be differentiated from HbC and HbE because all have identical migration speeds.

T F 9. Hemoglobin S cannot be separated from HbD by electrophoresis, but they can be differentiated by the sickling test, alkali denaturation test, and citrate agar electrophoresis.

T F 10. In HgB electrophoresis the unknown hemoglobin is identified by comparing its speed of migration with that of a known control.

T F 11. Citrate agar gel electrophoresis is used in diagnosis of thalassemia minor.

T F 12. The medium of choice for routine serum and hemoglobin electrophoresis is starch block.

T F 13. Two methods of preparing hemolysates are (1) by washing red cells with saline, then adding equal amounts of water and 0.4 volume of toluene; and (2) by freezing, then thawing.

413. Identify the principle and processes used in chromatography.

Chromatography. Different solutes move through absorption media at different rates and can therefore be separated from each other. This principle is the basis of chromatography. Although many investigators have contributed to the development of chromatography, principles applied today are essentially those developed by two early investigators, David Talbot Day (1859-1925) and Mikhail Tswett (1872-1919). Dr. Day was a geologist who, while working for the U.S. Government, observed the various colors of mineral samples. He attributed the color layers to "fractional filtration." He demonstrated that when crude oil was passed through finely pulverized earth, the first fraction was different from the second fraction; the second was different from the third, and so on. At approximately the same time that Day carried on his experiments in the United States, an Italian-born Russian physical chemist, Professor Mikhail Tswett, was working with pigments in leaves. He observed that when a petroleum ether extract was filtered through a column of absorbent material, the pigments which were dissolved in the solution were resolved according to their adsorption sequence as colored zones throughout the medium. Although separation today often includes colorless substances, the name chromatography is retained.

In the basic process of chromatography, a solution of small amounts of closely related substances in a suitable solvent is moved past a stationary phase that will adsorb different substances in varying degrees. As the solution moves past the stationary phase, the rates of flow of the more strongly adsorbed substances will be retarded the most, and the substances that are not adsorbed at all will move directly with the solvent front. Separation is effected because sample components are preferentially retarded by the stationary phase. If the action of the stationary phase depends solely on the adsorbing power (electrostatic attraction of the sample by "active" sites of the absorbent) of the solid, the process is called *adsorption chromatography.* If the stationary phase is an inert solid coated with a thin layer of adsorbed liquid so that the separation depends on the partition coefficient of the substances being separated between the solvent and the adsorbed immiscible liquid, this process is called *partition chromatography.* In other words, the solid absorbent is replaced by a stationary liquid that is normally only partially miscible with the flowing liquid. *Ion-exchange chromatography* is a refined process of the partition principle in which the stationary phase is a charged resin. If the sample is introduced in a charged state, it will be retarded according to its ability to displace the inorganic ions from the resin. *Molecular sieve chromatography* refers to selective exclusion of sample molecules by the porous stationary phase depending on the size and shape of the molecules. This is also called *gel filtration* when porous material is a gel which is supplied in a range of particle sizes suitable for many different applications. Both ion-exchange and gel-filtration chromatography are most commonly done in columns. All of these processes may occur to some extent in many chromatographic methods, but one is usually chosen to predominate.

Exercises (413):

Match the following by placing the letter of the column B item beside the number of the column A item which most nearly describes it. Each element in column B may be used once.

Column A

____ 1. The separation of mixtures by the use of their different absorptive properties in a common medium.

____ 2. A process in which the solid absorbent is replaced by a stationary liquid that is normally only partially miscible with flowing liquid.

____ 3. A refined process of the partition principle in which the stationary phase is a charged resin.

____ 4. A process in which there is separation of mixtures by the use of their different absorptive properties in a common medium.

____ 5. Refers to selective exclusion of sample molecules by the porous stationary phase depending on the size and shape of the molecules.

____ 6. Selective exclusion of the sample molecules by the porous stationary phase in which the porous material is a gel.

Column B

a. Adsorption chromatography.
b. Chromatography.
c. Partition chromatography.
d. Ion-exchange chromatography.
e. Molecular sieve chromatography.
f. Gel filtration.

414. Identify the given chromatographic techniques and the processes and reagents used.

Chromatographic Techniques. The previous discussion was on the general principles of the different major classifications of chromatography, rather than individual techniques. These techniques will be briefly presented.

Column chromatography. The technique first developed was that of column chromatography. In this technique, a stationary phase such as an ion-exchange resin, alumina, or silica gel in a finely divided form is placed in a column with a fritted disk or other device at the bottom to keep the stationary phase in place but allow the liquid to flow through. Usually the material is added to the column as a slurry in a solvent in order to obtain an even packing. The substances to be separated are placed on the top of the column in solution. The appropriate solvent is then allowed to flow through the column either by gravity or, in some cases, with the aid of applied suction or air pressure.

As the solvent flows through the column, the substances being separated will move down the column at different rates, depending on how strongly they are adsorbed by the column material. Eventually they will all be eluted. By successive collection of aliquots of solvent, the substances being separated will be in different fractions of the eluate and can be analyzed by the usual chemical methods. Usually, in order to obtain a separation in a reasonable length of time, a number of different solutions are applied to the top of the column in succession to elute different fractions. With aqueous solutions the differences are usually in pH or in buffer or salt concentration. With organic solvents the differences are usually increasing amounts of a polar solvent (such as ethyl alcohol) in a nonpolar solvent (such as benzene).

Since the order in which the substances will be eluted from the column cannot be accurately predicted in advance, the best conditions for separating any particular compounds must be determined experimentally. Column chromatography is often used to separate a particular substance from other impurities that would interfere with its chemical determination. The mixture is applied to a column. The desired substance may be strongly adsorbed and the impurities removed by the passage of a solvent through the column. Then by using a different solvent, the desired substance may be eluted in relatively pure form for analysis. Less commonly, the impurities may be strongly adsorbed and the desired substance eluted first from the column.

Paper chromatography. A second technique is paper chromatography. In this method the stationary phase is a strip of absorbent paper similar to filter paper. A small amount of solution containing the substances to be separated is applied near one end of the paper strip and the solvent evaporated. If the paper strip is suspended so that the end nearest the point of application of the substances is dipped into a suitable solvent, the solvent will wet the paper and gradually rise through the strip by capillary action. The flow of the solvent will carry the substances along with it at varying rates, depending on the degree to which they are absorbed by the paper. After the solvent has risen to the desired height in the paper, the strip is removed and the solvent is evaporated. The strip can then be sprayed with or dipped into a reagent that will give a color reaction with the substances being determined. If these are separated, a series of distinct spots on the paper will correspond to the individual compounds. The size of the spot or intensity of its color will be roughly proportional to the amount of the particular substance present. These may be compared with the spots obtained with known amounts of standards run similarly.

Thin-layer chromatography. Another variation is thin-layer chromatography (TLC). The application of TLC to the separation and identification of many substances is becoming increasingly useful in the clinical laboratory. It is less cumbersome and much more rapid than paper chromatography. Some general directions applicable to most determinations when supplemented by specific modifications for the particular compounds being studied will be discussed.

The adsorbents used in TLC are spread in a thin layer on an inert plate of glass, polyester plastic, or aluminum, or can be impregnated in a glass fiber network to make a semirigid sheet. The most commonly used adsorbents are silica gel, silicic acid, alumina, and cellulose powder. A wide variety of TLC media are available commercially. They may be purchased to meet a variety of needs: with a fluorescent indicator to aid in identification of some compounds; with various thicknesses of adsorbent; and with impregnated substances, such as borate, silicate, and phosphate to modify the character of the adsorbent. Individual requirements must be determined by the character and amount of materials to be studied and by the degree of experience and individual preference. The flexible sheets have been easiest to work with and quite adequate for precise quantitative work.

Gas chromatography. A recent development is gas-liquid chromatography, often simply termed "gas chromatography." In this method the substances being analyzed are in the gaseous state. The "solvent" that carries the material through the column is a stream of inert gas, commonly helium or nitrogen. The columns contain an inert material such as crushed firebrick on which is absorbed a thin layer of a liquid having a very low vapor pressure. The columns are usually of small diameter and many feet in length (often coiled to conserve space). The effluent gas from the column is automatically analyzed by one of several methods and the result recorded on a strip chart. Unless the substance being determined is a gas at room temperature (O_2, CO_2, or CO extracted from blood, for example), all the parts of the apparatus must be heated to well above room temperature to keep the material being analyzed in the gaseous state. The material to be analyzed is usually injected into the column in a small amount of volatile solvent. Since only very small amounts of material are required, a high enough concentration in the gaseous phase can be obtained for many substances without undue heat, which might decompose them. If the substance to be determined is not sufficiently volatile, it may be converted into a more volatile derivative before analysis. With the use of different column packings, a wide variety of biologic materials can be determined. Although the actual determination in the gas chromatography may be relatively simple, it often requires considerable time and effort to separate the material from other interfering substances and to prepare a suitable volatile derivative. Since different types of material may require different column packings, the gas chromatograph is most useful when it can be used for the analysis of a relatively large number of similar compounds (such as urinary steroids).

Exercises (414):

Match each of the column B items with the statement in column A which most nearly describes it. Each column B item may be used once or more than once.

	Column A	*Column B*
___	1. The appropriate solvent is allowed to flow through the column after the sub-	a. Column chromatography.
		b. Thin-layer chromatography.
		c. Paper chromatography.
		d. Gas chromatography.

Column A

stances to be separated are placed on the top of the column.

____ 2. Is often used to separate a particular substance from other impurities that would interfere with its chemical determination.

____ 3. The strip used can be sprayed with or dipped into a reagent that will give a color reaction with substances to be determined.

____ 4. Absorbents used are spread in a thin layer on an inert plate of glass, polyester, plastic, or aluminum; can be impregnated to make a semirigid sheet.

____ 5. Distinct spots will correspond to the individual compounds; the size or intensity of its color will be roughly proportional to the amount of the particular substance present.

____ 6. The most commonly used absorbents are silica gel, silica acid, alumina, and cellulose powder.

____ 7. Flexible sheets have been easiest to work with and quite adequate for precise quantitative work.

____ 8. The solvent that carries the material through the column is a stream of inert gas, commonly helium or nitrogen.

____ 9. All parts of the apparatus must be heated to well above room temperature.

415. Define serum iron and unsaturated iron binding capacity (UIBC); cite the significance of these determinations and the advantage of the methods given for analysis.

Serum Iron and Iron-Binding Capacity. The iron reserves of the body are stored as ferritin in the liver, spleen, bone marrow, and mucosal cells of the intestine. Absorption of iron is limited by the iron-binding capacity of the intestinal mucosa. Intestinal absorption, erythropoiesis, hemoglobin catabolism, and siderophilin metabolism are all factors which influence the serum (or plasma) iron level. Low values are found in hypochromic types of anemias and often individuals suffering from various infectious diseases. High values have been found in those anemias characterized by decreased hemoglobin formation not due to iron deficiency, such as pernicious anemia.

A clinically significant iron concentration is present in plasma or serum in excess of that normally present as hemoglobin. The normal values reported vary with the quantitative method, but are in the range of 65 to 175 μg percent. Earlier methods reported lower values in females but more recent studies found no sex difference. This nonhemoglobin serum iron is known as transport iron, acid-soluble iron, loosely bound iron, or protein-bound iron. Nonhemoglobin iron is not the total iron reserves but rather iron in transit from one part of the body to another. Siderophilin (transferrin) is a β globulin which loosely binds nonhemoglobin iron during its transport. Only about one-third of the available siderophilin is normally bound to iron. This is the serum iron. The remaining unsaturated siderophilin constitutes 60 to 70 percent of the available siderophilin. This unsaturated siderophilin is the unsaturated iron-binding capacity (UIBC) or latent iron-binding capacity (LIBC). Serum iron plus the UIBC is the total iron-binding capacity (TIBC) of serum. Serum iron expressed as a percentage of total iron-binding capacity is the percent saturation.

$$\frac{\text{Serum iron}}{\text{TIBC}} \times 100 = \text{percent saturation}$$

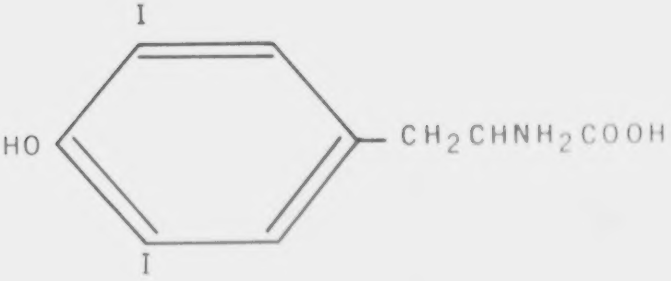
Quantitation of serum iron involves first splitting off the iron from its carrier protein. This is usually done with hydrochloric acid. Then the proteins are precipitated with acetic acid, and the supernate is reacted with nitric acid and potassium thiocyanate for color development. Another method uses hot trichloracetic acid, which simultaneously releases the iron and precipitates protein. This method continues with reduction of iron using hydrazine sulfate and color development with sulfonated bathophenanthroline.

Exercises (415):

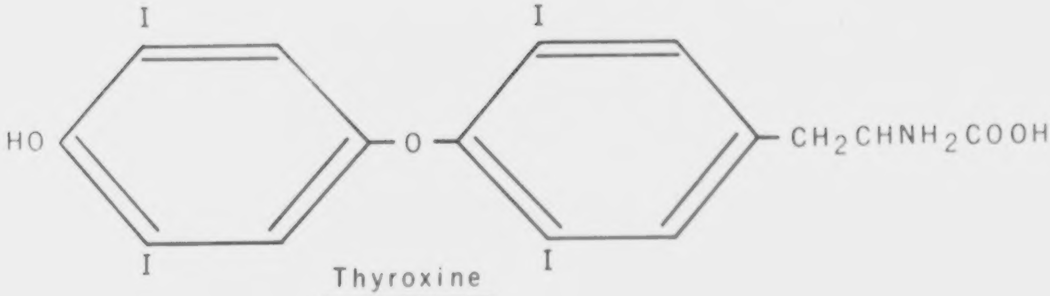
1. What is serum iron?

2. In what types of diseases are low serum iron values found?

3. What is the given normal range for quantitative serum iron?



3,5-Diiodotyrosine



Thyroxine

Figure 3-4. Structural formulas of diiodotyrosine and thyroxine.

4. What is the remaining unsaturated siderophilin called?

5. What are the constituents of the total iron-binding capacity?

6. What is the advantage in using hot trichloroacetic acid in the procedure for serum iron?

416. Indicate the significance of PBI, constituents of PBI, methods of analysis, reagents, normal values, and sources of error.

Protein-Bound Iodine. Iodine exists in serum as thyroxine (80 to 90 percent), inorganic iodine (10 percent), diiodotyrosine, triiodothyronine, and diiodothyronine. Thyroxine is the compound of interest in the evaluation of metabolic processes involving the thyroid gland. Diiodotyrosines are assumed to be precursors of the thyroxine molecule. Note the similarities of their structural formulas in figure 3-4. Thyroxine circulates in loose association with a protein in the blood plasma; thus, the term protein-bound iodine (PBI).

Inorganic iodide existing in the blood must be separated from the PBI before analysis. This was formerly done by precipitating the proteins (and PBI) and washing the precipitate to remove inorganic iodide. The precipitate is then used for analysis. A simpler method now uses an ion-exchange resin that will absorb the organic iodide but not the PBI. The serum is analyzed after the resin is separated. The analysis is done by first destroying all organic matter either by alkaline incineration or by wet digestion with chloric acid. The resulting inorganic iodide is determined colorimetrically.

So far in PBI analysis we have considered methods for separating the organic iodide and digestion of the organic residue to release inorganic iodide. The final general consideration is quantitation of released iodine. All methods in use at present employ the ceric-arsenious acid reaction for colorimetric determination of iodine from PBI. This reaction involves reduction of yellow-colored ceric ions to colorless cerous ions by arsenious acid through the catalytic effect of iodide ions. There is a linear relationship between reaction time and iodide concentration. The *rate* of reaction or *change* in optical density rather than a final stable optical density determines the concentration thus obtained. Timing intervals between O.D. readings become an important factor in this colorimetry. For this reason, the number of assays determined at one time depends upon the manual dexterity of the technician.

Normal values are generally 4 to 8 $\mu g/100ml$, but this varies somewhat depending upon the technique and geographic area.

Limitation of the PBI. It is impossible to discuss the PBIs without mentioning the ever-present problem of contamination. Probably no other test is more prone to contamination than the PBI. Contamination may come from the patient. This exogenous iodine may result from radiopaque dyes used in cholecystography, urography, or myelography; iodinated amoebicides; vaginal suppositories containing diiodohydroxyquin and iodothiouracil; tinctures of iodine, merthiolate, mercurochrome; or the use of "all weather" suntan lotions containing iodine. Inorganic iodine contamination may also result from technicians who collect specimens after working with Gram's stain or Lugol's solution. Water used in PBI analysis is often a source of contamination. It must be glass redistilled and deionized before use. All glassware must be scrupulously cleaned in chromic acid and thoroughly rinsed.

Other limitations of the PBI as a thyroid function test include the following:

a. Mercurial diuretics and gold therapy may inhibit iodine catalysis in the arsenious acid ceric ammonium sulfate system; however, this is not a problem with the alkaline ash procedure.

b. The PBI—and other measurements of serum hormone—may be invalidated by an increase or decrease in the T4:T3 ratio.

False high PBI results frequently are due to endogenous or exogenous iodine contamination. The problems caused by iodine interference were recognized early and led to development of more specific methods for measuring thyroid hormone in serum. These will be discussed later in this chapter.

Automated analysis. The digestion of organic material by a mixture of sulfuric acid, nitric acid, and perchloric acid has been widely employed in the Technicon procedure for automated determination of serum PBI. Complete digestion requires only 3 minutes at 280° C, and the conditions of the automated system are extremely well controlled. These factors confer acceptable accuracy on the procedure.

Exercises (416):

Indicate whether each of the following sentences is true (T) or false (F) and correct those that are false.

T F 1. Iodine exists in serum as inorganic iodine to about 80 to 90 percent.

T F 2. Thyroxine is a compound of interest in the evaluation of metabolic processes involving the thyroid gland.

T F 3. Inorganic iodide circulates in loose association with a protein in the blood plasma, thus deriving the term "protein-bound iodine" (PBI).

T F 4. Inorganic iodide existing in the blood must be separated from the PBI before analysis.

T F 5. A simpler method for separation of inorganic iodide requires precipitating the proteins and washing the precipitate.

T F 6. Analysis is done by first destroying all organic matter either by alkaline incineration or by wet digestion with chloric acid.

T F 7. Manual methods used for PBI employ the ceri-arsenious acid reaction for colorimetric determinations.

T F 8. The type of reaction or stability of optical density rather than the final optical density determines the concentration obtained for PBI.

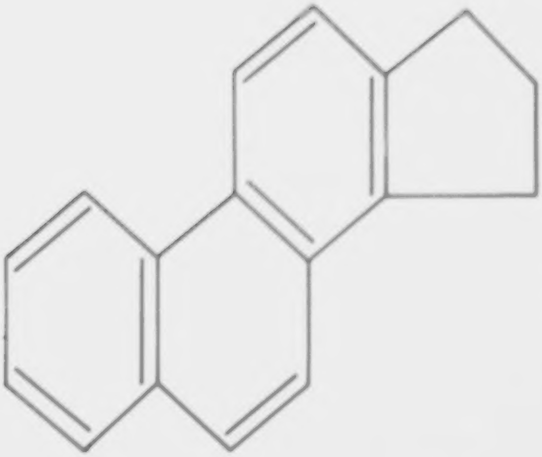


Figure 3-5. Structure of the cyclopentenophenanthrene ring.

T F 9. Normal values for PBI are 0.4 to 0.8 μg/100 ml.

T F 10. Contamination of PBI can result from tinctures of iodine, merthiolate, mercurochrome, and suntan lotions containing iodine.

T F 11. Mercurial diuretics and gold therapy may inhibit iodine catalysis in the arsenious acid ceric ammonium sulfate system, a problem also with the alkaline ash procedure.

T F 12. The automated Technicon procedure requires the digestion of organic material by a mixture of hydrochloric acid, chloric acid, and trichloroacetic acid.

417. Point out briefly the method of analysis and procedure for serum thyroxine (T_4), reagents, normal volume, and sources of contamination.

Determination of Thyroxin (T_4) by Column. The first significant improvement was the chromatographic thyroxine, or "T_4 by column." This new measurement was developed in the early 1960's; by 1966 an automated version had become widely available and largely replaced the PBI. In analogy to the PBI, many laboratories report the T_4 by column as thyroxine iodine; this may be multipled by 1.5 to obtain serum thyroxine. The main thyroid hormone, T_4, together with the smaller amount of triidothyronine (T_3), is separated from serum by the use of column chromatography on an ion-exchange resin. The solution of the serum in dilute ammonia with a pH 10.0-11.0 is placed on the column wherein the column is washed with water and then with acetate buffer and dilute acetic acid. The T_4 is then eluted from the column with a stronger solution of acetic acid (pH 1.4). The eluate is collected in two fractions. Usually the first will contain at least 70 percent of the T_4 (iodine). If the second fraction contains more than 30 percent of the total iodine, this usually indicates some type of contamination from other organic iodides.

If the acetic acid solution containing the T_4 were treated with bromine water, it could then be used directly in the ceric-arsenite reaction without the necessity of wet ashing and this reaction reduces contamination from contrast media containing iodine. The abbreviation $T_4I(C)$ is recommended for T_4 by column reported as thyroxine iodine (normal range about 3.2 to 7.2 μg/dl). The abbreviation $T_4(C)$ is recommended for T_4 by column reported as serum thyroxine (normal range about 5. 0 to 11.0 μg/dl).

The following types of iodine contamination are eliminated: (1) endogenous contamination, including MIT, DIT, and abnormal iodinated protein; (2) exogenous iodide contamination; and (3) exogenous iodine contamination from a number of radiographic contrast media, including Chlografin, Dionosil (low levels), Hippuran, Hypaque, Miokon, Orabilex (low levels), Oragrafin, Pantopaque, Renografin, Salpix, Skiodan, and Urokon. Although this method does not completely solve the problem of iodine contamination, much of the interference is either eliminated or detected.

This represents a significant improvement over the PBI. Procedures that do not require digestion were originally designed as manual methods but recent modifications permit analysis of the eluate by the Auto Analyzer.

Since the T_4 by column method offers greater specificity than the classic PBI method, it has become the method of choice in most laboratories that perform such procedures. The increased specificity is due to the elimination of many troublesome iodine-containing organic compounds in the first wash and the retention of inorganic iodide by the column.

A number of tests use T_3 or T_4 labeled with radioactive iodine for estimation of thyroid function. Such an example is the T_4, a radioisotopic procedure as described by Murphy and Pattee in 1964. This method, unaffected by contamination with iodine (organic or inorganic, as well as mercury), has gained widespread acceptance. This procedure will be discussed briefly in the next section on radioisotopes.

Exercises (417):

1. What method of analysis is used to separate serum thyroxine, (T_4) and triiodothyronine (T_3) from serum?

2. Initially, what reagent is used to dilute the serum to a pH of 10.0-11.0 before placing it on the column?

3. What reagent is used to elute the T_4 from the column?

4. What is collected in the first eluate and what percentage? The second eluate and what percentage?

5. What process may be used to produce a reaction in order to reduce contamination from contrast media containing iodine?

6. What is the normal range for T_4 by column abbreviated as $T_4I(C)$ (thyroxine iodine)?

7. What is the normal range for T_4 by column abbreviated as $T_4(C)$ (serum thyroxine)?

8. What two types of contamination are eliminated by this method?

418. Match terms related to radioisotope measurements with the appropriate definitions or procedures.

Radioisotopes. Radioactive isotope measurements have now achieved an important place in the clinical laboratory as an aid in the diagnosis of a variety of pathological conditions, such as abnormal functioning of the thyroid and pancreas. These tests are usually performed by specially trained personnel in referral laboratories. As a regular laboratory technician, you do not need to know the detailed procedures for these measurements, but you do need to understand the principles. Also, since the laboratory must obtain specimens for analysis and referral, you should be able to provide some assistance in this area.

As discussed in a previous chapter, an isotope is a chemical element having the same atomic number (the same number of nuclear protons) as another element but possessing a different atomic mass (a different number of nuclear neutrons). A radioisotope is an isotope that emits electromagnetic radiation. Radioisotopes occur naturally, or they can be produced by bombardment of a common chemical element with high velocity particles from a chain-reacting pile or cyclotron. This causes neutrons, protons, deuterons, or alpha particles of the atom to become active and the isotope to become unstable. These isotopes decompose at a rate that is fixed and definite for the particular isotope but differs from the rates for other radioisotopes. This rate of decay or decomposition is known as the half-life. The half-life is the time required for one-half of the atoms of a radioisotope to decay. I^{135}, for example, has a half-life of 60 days. This means that in 60 days one-half of its atoms will have decomposed and will have become stable (no longer emit radiation). In another 60 days one-half of the remaining radioisotopes will have decayed and become stable, and so on.

Measurement of radioisotopes. The radiation of radioisotopes, which consists of beta particles or gamma rays, can be measured by means of an activated sodium iodide crystal. When particles from a disintegrating atom enter the crystal and are absorbed, a small flash of light is produced, and this light is detected by a photomultiplier tube attached to the crystal. The resultant electric pulse from the phototube is amplified and counted by electronic means. The total number of pulses detected per unit of time is a measure of the radioactivity of the sample.

The use of radioactivity in analysis depends on the fact that the radioactive atoms of a substance, such as iodine, react chemically just as the nonradioactive iodine atoms do, but the unstable elements can be readily detected by means of their radioactivity. I^{131}, which has a half-life of 8 days, is very suitable for radioactivity studies in man.

Radioiodine uptake and excretion tests. Five to $25\mu c$ of radioactive iodine (I^{131}) is given to the patient orally, and readings are taken at 6- and 24-hour periods, measuring the amount of radioactivity entrapped by the thyroid. The radioactive I^{131} uptake is affected by the ingestion of both organic and inorganic iodides, steroids, and other drugs.

The urinary excretion of radioactive iodine is also used in the diagnosis of dysfunction of the thyroid. A 24-hour urine test is required. Normally 30 to 70 percent of the dose is excreted in the 24 hours after administration. Less is excreted in hyperthroidism and more in hypothyroidism. The excretion test is less reliable than the uptake test because dependable results are obtainable only if renal function is unimpaired (a fact not easily proven).

Radioactive T_3 red cell uptake test. Red cell uptake is an in vitro test with the advantage that no radioactive material need be administered to the patient. The patient's blood is drawn and mixed with T_3 (radioactive triiodothyronine) and allowed to stand. The percentage of T_3 that combines with the surface of the patient's red cells is measured. Normally 25 to 35 percent combines with the red cells. If the thyrobinding protein is saturated with thyroxine (T_4), as in hyperthyroidism, most of the radioactive T_3 will combine with the red cells, and the T_3 uptake will be above 40 percent. If only a small amount of throbinding protein is bound to thyroxine, as in hypothyroidism, most of the radioactive T_3 will combine with the thyrobinding protein and less with the red cells. Red cell uptake is useful in patients who have recently ingested iodides or had radiocontrast studies and for whom PBI values would be inaccurate. Neither organic nor inorganic iodine distorts this procedure. Some techniques utilize a resin sponge instead of red cells.

T_4 (by isotope). The T_4 by isotope, or Murphy-Pattee, involves a process of competitive binding. The patient's serum (one milliliter) is mixed with 2 ml of ethanol and allowed to stand. The alcohol extracts most of the thyroxine in the serum. The extract is added to a standard solution containing TBP-thyroxine I^{125}. While the solution stands, the patient's thyroxine competes with the radioactive thyroxine for binding sites on the thyroid-binding protein (TBP) and displaces some of the radioactive thyroxine in a free state. At this point, a resin sponge is placed into the solution to combine the thyroxine I^{125}. The resin is separated, washed, and the radioactivity measured by a counter, as previously described.

Triolein uptake. For studies of malabsorption, triolein combined with I^{131} is given to the patient. Plasma, urine, and feces from the patient are then scanned for radioactivity. Normally less than 2 percent of the "tagged" triolein is excreted in the feces in the following 48 hours, and plasma levels of 12 percent or higher are obtained in 6 hours. In malabsorption syndrome less triolein is absorbed so that the fecal level is higher and plasma level is lower. Patients having chronic pancreatitis may also have diminished absorption of radioactive triolein. It is reasoned here that these individuals lack the enzymes to convert triolein to oleic acid and glycerol. To differentiate between these two conditions, olein acid "tagged" with I^{131} is given the patient. This is easily absorbed in chronic pancreatitis, but is excreted by individuals with malabsorption syndrome.

Blood volume. In these determinations a specific amount of serum albumin labeled with a specific number—20 or so—of μc (microcuries) of radioactive iodine is injected intravenously. Sufficient time should be allowed to circulate through the body (approximately 10 minutes). A blood sample is then taken and analyzed for radioactivity. The blood volume can now be determined indirectly by dividing the number of radioactive units injected by the dilution in milliliters per minute (decreased radioactivity). Red cells tagged with Cr^{51} may be used in a similar manner. Although using the cells is more accurate, it is very time consuming. Both methods can be used simultaneously to achieve the best results. This procedure is of value in the differentiation of hemorrhagic shock from other forms of circulatory failures of the peripheral system. Cr^{51} studies are also helpful in following cases of acute renal failure, gastrointestinal bleeding, intestinal obstruction, and the diagnosis of early congestive heart failure.

Blood cell survival can also be determined with the use of Cr^{51}. Blood is withdrawn from the patient, tagged with the isotope, and reinjected. Serial blood samples are assayed for radioactivity over a period of 2 weeks. A red cell half-life below 26 days is suggestive of hemolytic anemia.

Exercises (418):

Match each procedure or term in column B with the appropriate principle or technique to which it closely relates in column A. Each item in column B may be used once or not at all.

Column A

____ 1. Valuable in differentiating hemorrhagic shock from other forms of circulatory failures of the peripheral system.

____ 2. The patient's serum is mixed with 2 ml of ethanol to extract the thyroxine in the serum as the initial process in competitive binding.

____ 3. Following the administering of this radioactive compound, plasma, urine, and feces from the patient are scanned for radioactivity.

____ 4. Five to 25 μc of radioactive iodine (I^{131}) is given to the patient orally, and readings are taken at 6- and 24-hour periods measuring the amount of radioactivity entrapped by the gland.

____ 5. The patient's blood is drawn and mixed with T_3 (radioactive triiodothyronine) and allowed to stand.

Column B

a. Half-life.
b. Photomultiplier tube.
c. Inorganic and organic iodine.
d. Radioiodine uptake.
e. Radioactive T_3 red cell uptake.
f. T_4 or Murphy-Pattee (by isotope).
g. Triolein uptake.
h. Blood volume.
i. Phosphates.
j. Eight days.

Column A

The percentage of T_3 that combines with the surface of the patient's red cells is measured.

____ 6. Interfering substances of radioactive I^{131} uptake studies.

____ 7. Collects and amplifies small flashes of light emitted by disintegrating atoms as in radioactive isotopes.

____ 8. Period of time required for approximately half of a given number of atoms of a radioactive isotope to decompose into a stable state.

3-2. Hormones

A hormone is a chemical substance produced by an endocrine gland, for example, a gland of internal secretion. One exception to this definition is the placenta which produces hormones, but which is not—strictly speaking—an endocrine gland. You are undoubtedly aware that it is placental gonadotropin which you measure in pregnancy testing. The purpose of a hormone is to control the functional activity of another part of the body. For example, certain hormones secreted by the pituitary gland control thyroid activity. The effect of hormones is measured indirectly in the laboratory in practically every test that is performed. For example, a blood glucose result reflects the activity of the adrenal and pituitary glands. This was explained in a previous chapter concerning glucose. The purpose of the present discussion, however, is to point out that a more direct approach is possible by studying and evaluating endocrine functions. Specifically, each hormone or group of hormones can be directly assayed in the laboratory. Two major classifications discussed here are steroid hormones and pituitary hormones.

419. Indicate whether given statements correctly reflect the significance of the assay of steroid hormones, methods of analysis, and specimens required.

Steroid Hormones. The hormones known as steroid hormones are characterized by the ring configuration shown in figure 3-5. This particular 17-carbon atom structure is known as the cyclopentenophenanthrene ring. (Note the similarity to cholestrol described earlier in this course. In fact, all adrenal steroid hormones are derived from cholesterol.) The assay of steroid hormones is of considerable medical interest in the study of certain functional disorders. Unfortunately, some of these analyses are beyond the capability of the average medical laboratory. A clear understanding of these tests and their limitations is essential for proper interpretation of your technical responsibilities. The following is a brief review of steroid hormones and some methods of analysis available to clinical laboratories.

Estrogens. Estrogens include estradiol, estrone, equilenin, and estriol. An assay of the individual estrogens is seldom of clinical importance; hence, total estrogens only are considered. Estrogens are formed in the ovaries, the adrenal cortex, and the placenta. They are secreted mainly in the urine as glucuronidates and sulfates. Estrogens are usually assayed by vaginal cytologic examination, which is an estimation of the estrogen activity. Bioassay, colorimetric, and fluorometric methods are either nonspecific or too involved to be performed in a clinical laboratory.

Urinary estrogen is chiefly estriol with smaller amounts of estradiol and estrone. The most significant clinical value of estrogen measurement may be in the diagnosis of fetal distress during pregnancy. The estriol excretion usually increases progressively during the third trimester of pregnancy. Any sharp decrease in the amount of estriol excreted or a failure of the quantity of hormone excreted to rise progressively is evidence of a complicated pregnancy. Decreased estrogen levels lead to sexual immaturity in the female. Increased values are most commonly associated with tumors of the ovaries.

Usually a 24-hour urine sample is collected with no perservative added and kept under refrigeration. Since estrogen is excreted chiefly as glucuronides, these glucuronides should be hydrolyzed before extraction. This hydrolysis is done either enzymatically or by heating with acid.

Progesterone. Formed in the corpus luteum of the ovary, its concentration closely parallels the development, activity, and regression of the corpus luteum. It is also formed in the adrenal cortex as an intermediate compound in the biosynthesis of adrenal corticoids. The reduction product, pregnandiol (progesterone is always excreted as the glucuronide of pregnandiol in the urine), is the form which is normally analyzed. Pregnandiol is determined usually by column chromatography. Other methods of analysis are available but are not ordinarily performed in the usual clinical laboratory. Lower values which are excreted during pregnancy are associated with threatened abortion or toxemia of pregnancy. In suspected cases of congenital adrenal hyperplasia, the assay of an intermediate product, pregnanetriol, may be important in differentiating this disease from other adrenal cortical disorders.

Androgens. The testicular androgen, testosterone, is not normally excreted in the urine but is metabolized to androsterone, epiandrosterone, and 11-hydroxyandrosterone (the prefix *iso* sometimes used instead of *epi*) which are normally found in the urine as sulfates and glucuronides. The remainder of the urinary androgens are androsterone and dehydroepiandrosterone of adrenal cortical origin. In males approximately one-third of the urinary androgens are formed in the testes and two-thirds are produced by the adrenal cortex. In females the androgens are formed solely in the adrenal cortex. Androgens are assayed clinically by the urinary 17-ketosteroid procedure. The method is not specific for androgens, however.

A number of chromatographic techniques are available for separation of testosterone from urinary and plasma extracts. These are thin-layer and gas chromatography. Reagent quantitation of testosterone may be determined by flame-ionization detection (gas-liquid chromatography), double-isotope derivative techniques, acid fluorescence, and competitive protein. In most clinical laboratories, the competitive protein binding technique is the method of choice.

Radioimmunoassay, a technique to be discussed later, is best to measure plasma or serum testosterone and dihydrotesterone levels as well as dehydroepiandrosterone (DHA) and other androgens. Urinary methods are not reliable indicators of androgen secretion because of the interconversion of androgens and dynamics of metabolism.

Exercises (419):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

T F 1. All adrenal steroid hormones are derived from cholesterol.

T F 2. Estrogens are formed in the pituitary, the adrenal medulla, and the placenta.

T F 3. Bioassay, colorimetric, and fluorometric methods are quite simple and specific for analysis of estrogens.

T F 4. Urinary estrogen is chiefly estriol with smaller amounts of estradiol and estrone.

T F 5. The most significant clinical value of estrogen is its use in the diagnosis of fetal distress during pregnancy.

T F 6. Decreased values of estrogens are associated with tumors of the ovaries.

T F 7. A 48-hour urine sample is required with no preservatives added and kept under refrigeration.

T F 8. Progesterone is always secreted as the glucuronide of pregnandiol in the urine.

T F 9. Progesterone is the reduction product and the form in which it is analyzed.

T F 10. Pregnandiol is determined by thin-layer chromatography.

T F 11. Higher pregnandiol values which are excreted during pregnancy are associated with threatened abortion or toxemia of pregnancy.

T F 12. Androgens are assayed clinically by the urinary 17-ketosteroid procedure.

T F 13. The best technique to measure plasma or serum testosterone and dihydrotestosterone levels as well as other androgens is the competitive binding technique.

T F 14. Testosterone is the most biologically active androgen.

420. Specify the method of analysis, procedures, reagents, significance, normal values, and sources of errors for 17-ketosteroids (18 KS), 17-ketogenic steroids (17-KGS), and 17-hydroxycorticosteroids (17-OH corticosteroids, 17-OHCS).

Andrenocortical Steroids. The corticosteroids are, from the physiological as well as the quantitative points of view, the most important group of adrenal steroids. These hormones of the adrenal cortex proper, or corticosteroids, consist of over 40 members which have been isolated, but only a few are biologically active. The corticosteroids help regulate protein, carbohydrate, and fat metabolism, and water and electrolyte balance. The above classes of hormones are formed from the same basic compounds (acetates) which form cholesterol. Cholesterol is thought to be converted to the adrenal cortical hormones by one of several complicated enzymatic pathways. These hormones are all basically similar, and their separation sometimes depends upon lengthy extraction procedures with subsequent colorimetric analysis. Procedures for 17-ketosteroids and hydroxycorticosteroids are normally used to assay adrenal cortical hormones.

Measurement of Urinary Steroids. Urinary steroids can be conveniently divided into three major groups: 17-ketosteroids (17-KS), 17-ketogenic steroids (17-KGS), and 17-hydroxycorticosteroids (17-OH corticosteroids, 17-OHCS). It is rather unfortunate that these terms appear to be so similar, because the substances which they measure are quite different. Shown in table 3-2 is a list of steroids which are of particular interest in an evaluation of adrenal cortical function and which react in one of the common corticoid assays.

17-Ketosteroids. The procedure used for *17-ketosteroids* assays the androgens (androsterone, epiandrosterone, dehydroepiandrosterone) and the nonandrogen etiocholanolone by the Zimmerman reaction. In the Zimmerman reaction, 17-ketosteroids produce a red color in alkaline solution with m-dinitrobenzene. Urine sugar levels of more than 1+ will inhibit this reaction, causing low values. The 17-ketosteroids can be further divided into alpha and beta fractions, the beta 17-ketosteroids being precipitated by digitonin, whereas the alpha are not. The alpha 17-ketosteroids normally predominate (85 to 95 percent), consisting of androsterone and etiocholanolone, while the beta fraction is present in only small amounts and consists of epiandrosterone and dehydroepiandrosterone. Normal urine values for neutral 17-ketosteroids are as follows. Children: daily output in the urine for children to 6 years of age is less than 1 mg. Values rise gradually until adult levels are reached at 12 to 18 years of age. Adult female: the usual range for the adult female is 5 to 15 mg/24 hours. Adult male: 8 to 25 mg/24 hours. Values for 17-ketosteroids are diminished in hypopituitarism, pituitary tumors, hypogonadism, Addison's disease, and certain other conditions. Values are elevated in cases of testicular tumors, adrenal hyperfunction, and following endocrine therapy.

A 24-hour urine specimen is collected with such preservatives as hydrochloric acid, toluene, or boric acid. Some methods require no preservative except that the specimen be kept cold during collection until analysis.

17-Ketogenic steroids. This is a term similar to that of 17-ketosteroids, but having a completely different set of compounds. The term 17-ketogenic steroids refers to compounds having 21 carbon atoms and a hydroxyl group at C-17. This hydroxyl group can be converted to a ketone group by oxidation, which also removes the side chain of C-20 and C-21 regardless of its nature. The Zimmerman reaction (meta dinitrobenzene) for 17-ketosteroids is applied to final extracted products. Since reduction with borohydride is followed by oxidation with bismuthate or periodate, the original 17-ketosteroids are no longer included in the final caloried products of the reaction. Thus, a more total estimation of 17-hydroxycorticoids and adrenocortical function is obtained with 17-ketogenic steroid assay. The normal values are considered similar for both male and female: 5 to 20 mg/24 hours. A 24-hour urine sample is kept refrigerated during collection until taken to the laboratory for analysis.

17-Hydroxycorticosteroids. 17-Hydroxycorticosteroids can be a misleading term since not every 21 carbon compound with a 17-hydroxyl group is included. The steroids included are those with 21 carbons and a dihydroxyacetone side chain. This combination of hydroxyl groups at C-17 and C-21, and a ketone group at C-20 reacts with phenylhydrozene to form a yellow compound. This is the Porter-Silber reaction, and these steroids are often called *Porter-Silber* chromogens. Although the 17-hydroxycorticosteroid determination is a sensitive index of adrenocortical function, the 17-ketogenic steroid assay is more sensitive. The 17-ketogenic steroid assay is especially valuable in adreno-genital syndrome and rare examples of Cushing's syndrome. Normal values are (adult males), 4 to 12 mg/24 hours, and (adult females) 2 to 6 mg/24 hours, with slight variation depending on the method used to extract the compounds. Either high protein or urine sugar levels greater than 1+ will interfere with Porter-Silber reaction for 17-hydroxycorticosteroids.

Other interfering substances. Spironolactone, meprobamate, and a number of antihypertensives, tranquilizers, and other drugs interfere with the reaction of the 17-KS. Penicillin has been reported to be an interference in the 17-KS and 17-KGS analyses.

Automation in Steroid Analysis. Although the methods discussed may include may nonautomated techniques, the trend in the direction of automated steroid analysis must not be overlooked. The basic principles are included in the given methods. However, many laboratories may have the capability of using more automated techniques available. Two main areas of approaches or methodologies are automation of multiple steroid tests and gas chromatography. Of the two procedures, gas chromatography is more widely used and accepted as a means of quantitative analysis for steroids in biologic fluids.

TABLE 3-2
STEROID HORMONES RELATED TO CORTICAL FUNCTION

Steroid	*Synonyms*		*Reaction*
Corticosterone		Compound B	
11-Dehydrocorticosterone		Compound A	
11-Dehydro-17-hydroxycorticosterone	Cortisone	Compound E	17-Hydroxy, 17-KG*
17-Hydroxycorticosterone	Hydrocortisone Cortisol	C-21 Compounds Compound F	17-Hydroxy, 17-KG
11-Deoxycorticosterone		Compound Q	
17-Dydroxy-11-Deoxycorticosterone		Compound S	17-Hydroxy, 17-KG
Cortol			17-KG
Cortolone			17-KG
Tetrahydrocortisone			17-Hydroxy, 17-KG
Tetrahydrohydrocortisone			17-Hydroxy, 17-KG
Pregnantriol			17-KG
Aldosterone			
17-Hydroxy Progesterone			17-KG
17-Hydroxy Pregnanolone			17-KG

*17-KG = 17-Ketogenic steroids analysis
17-Hydroxy = 17-Hydroxycorticosteroids

Exercises (420):

Match the following by placing the letter of the column B item beside the number of the column A item or items that most nearly describe it. Each element in column B may be used once, more than once, or not at all.

Column A

____ 1. Help regulate protein, carbohydrate, and fat metabolism, and water and electrolyte balance.
____ 2. A reaction in which 17-ketosteroids produce a red color in alkaline solution with m-dinitrobenzene.
____ 3. Will be inhibited by a urine surgar of more than 1+.
____ 4. Normal values for adult females is 5 to 15 mg/24 hours.
____ 5. May be used as preservative for 24-hour urine specimen collected for 17-ketosteroids.
____ 6. Values are diminished in hypopituitarism, pituitary tumors, hypogonadism, and Addison's disease.
____ 7. Refers to compounds having 21 carbon atoms and a hydroxyl group at C-17.
____ 8. May be applied to final extracted products of 17-ketogenic steroid for analysis.
____ 9. Normal values, male and female: 5-20 mg/24 hours.
____ 10. A 24-hour specimen is kept refrigerated during collection until delivered to the laboratory for final analysis.
____ 11. Reaction in which a combination of hydroxyl groups at C-17 and C-21 react with phenylhydrazine to form a yellow compound.
____ 12. A more sensitive steroid assay than the 17-hydroxycorticosteroid determination.
____ 13. Is especially valuable in adreno-genital syndrome and rare examples of Cushing's syndrome.
____ 14. High protein levels greater than 1+ will interfere with this reaction.
____ 15. Will interfere in the analysis of 17-KS and 17-KGS.

Column B

a. Adrenocortical steroids.
b. 17-hydroxycortico steroids.
c. 17-ketogenic steroids (17 KGS).
d. Meprobamate and penicillin.
e. 17-ketosteroids (17 KS).
f. Porter-Silber reaction.
g. Zimmerman reaction.
h. Hydrochloric acid, toluene, or boric acid.
i. Estriol.
j. Ascorbic acid and aspirin.

421. State the significance of the pituitary hormones and the methods used in the evaluation of these hormones.

Pituitary Hormones. The pituitary gland is a small (average 1.3 × 1.0 × 10.5 cm), rounded grey body attached to the base of the brain. It is often referred to as the "master gland" because it regulates many other endocrine activities. The most important group of pituitary hormones in man are those which originate in the anterior lobe of the pituitary gland. Hormones of this group are known as the adenohypophyseal hormones. They include gonadotropic hormones, thyrotropic hormone, adrenocorticotropic hormone (ACTH), and somatotropic hormone. The gonadotropic hormone is responsible for activity of the ovaries in the female and spermatogenesis as well as androgen production in the male. Actually, the gonadotropic hormone is a group of hormones. Included in the group are the follicle-stimulating hormone (FSH), luteinizing hormone (LH), interstitial cell-stimulating hormone (ICSH), and luteotropic hormone (LTH).

The assay of pituitary gonadotropin may be accomplished collectively by classic bioassay procedures. However, recent advances in radioimmunoassay techniques have been more specific for FSH and luteinizining or interstitial cell-stimulating hormone (LH) or (ICSH) and have become available. Several methods have been employed for the bioassay of pituitary gonadotropin by using laboratory animals to show one or more of the following effects: (1) increase in weight of the uterus or ovaries, (2) production of corpora lutea, (3) increase in weight of seminal vesicles, and (4) effect upon estrus which is characterized by proliferation of the vaginal epithelium. The assay of chorionic gonadotropin, which is secreted by the placenta, is discussed under the heading of pregnancy tests later in this course. Results of gonadotropic studies are reported in mouse units. A *mouse unit* is the least amount of estrus-producing hormone which induces desquamation (sloughing) of the vaginal epithelium in a spayed mouse. The FSH stimulates the ovarian follicle to increase in size and to mature, while in the male it is significantly involved in the stimulation and maintenance of spermatogenesis. In the female LH causes ovulation and steroid production, such as estrogen and progesterone by the corpus luteum. LH stimulates the interstitial cells to produce androgens and estrogens. Pituitary gonadotropins (FSH and LH) require a 24-hour urine specimen by bioassay and serum or plasma by RIA. Normal adult range of urine is approximately 10-50 mouse uterine units (MUU) per 24 hours. The normal range for serum or plasma is thus:

Male:	4-25 milli-IU/ml
Female:	Premenopausal—4-30 milli-IU/ml Postmenopausal—40-50 milli-IU/ml Mid-cycle peak—2 × the baseline.

Exercises (421):

1. What hormone is responsible for activity of the ovaries in the female, and spermatogenesis and androgen production in the male?

2. What function does the FSH serve?

3. What is the function of LH?

4. What type of specimen is required for pituitary gonadotropins by bioassay?

5. What type of specimen is required for FSH and LH by RIA?

6. What is a *mouse unit* in the bioassay of gonadotropins?

422. Identify the principle and procedure of radioimmunoassay in terms of its uses and applications.

Principle and Procedure of Radioimmunoassay. The basic principle of radioimmunoassay (RIA) involves the specific binding of an antibody to an antigen which is to be measured. A radioactively labeled antigen competes with a known standard or unknown sample of unlabeled antigen for binding sites on the antibody molecules. The radioactivity bound to the antibody after incubation is closely related to the amount of unlabeled antigen present in the system.

Antibodies used in radioimmunoassay are produced in various designated animals by the injections, at repeated intervals, of a substance foreign to the animal. The substance, as an antigen, triggers the animal's immune responses to produce specific antibodies to the antigen.

After a series of suitable testings of the titer, specificity, sensitivity, and affinity, these animal serums may be used to measure the antigen in an in vitro assay.

RIA has become the method of choice for measuring hormones with a low circulatory concentration in blood. Over the past decade, much research has been done to expand the scope of RIA. It was earlier noted that this specific approach, if available for many substances, would be valuable in (1) saving time, (2) low cost, (3) highly accurate diagnosis, and (4) requirement for only small volumes of blood. Two assays of almost equal importance emerged about the same time; that is, competitive protein binding (CPB) and RIA. However, CPB is not usually as simple and specific as in the RIA.

As the technician, you must have a general idea of the procedural steps common to all radioimmunoassay. There are many kits and systems available for RIA; nevertheless, they all incorporate the following basic steps:

(1) Incubation of constant quantities of labeled antigen and antibody with a series of known standard amounts of unlabeled antigen and samples containing unknown amounts of that antigen.

(2) Separation of bound antigen from free antigen after the reaction has reached equilibrium. This may be accomplished by precipitation by a second antibody raised against the gamma globulin of the animal producing the first antibody, solid phase absorption of the free antigen or of the antibody, filtration, electrophoresis, chromatoelectrophoresis, and nonspecific precipitation of the antigen-antibody complex.

(3) Determination of the bound radioactivity. Bound, free, or both radioactivities may be counted. The instrumentation depends upon the isotope used.

(4) Calculation. A standard curve is drawn by plotting counts per minute (CPM) or per bound antigen (%B) against the logarithm of each known standard.

Use and Application of Radioimmunoassay. Presently, there are various methods available for the determination of hormones by RIA. A discussion of the background significance of the most commonly requested analysis will provide the technician with essential knowledge consistent with the enhanced quality of laboratory service which RIA systems introduce. The following tests are done by RIA.

Digoxin, digitoxin. Sample required is plasma or serum. Results are used to monitor digitalis therapy. Digitalis strengthens and normalizes the heart action, but must be carefully administered since toxic levels are easily reached.

Renin (Angiotensin I). Sample required is plasma

(EDTA). Renin helps diagnose and evaluate renal hypertension. Renin is produced by a damaged kidney and is responsible for inducing hypertension. Renin determinations help the physician evaluate the possibility of renal involvement in his patient's hypertension. Angiotensin II is a powerful vasoconstrictor, and while it stimulates aldosterone production, it also is responsible for hypertension of renal origin.

Aldosterone. Specimen required is 24-hour urine specimen. Aldosterone helps evaluate adrenocortical function. Aldosterone is an adrenal cortical hormone that is a strong salt retainer. Its increase can be caused by several factors, including salt depletion, cardiac failure, cirrhosis, nephrosis, and pregnancy.

Insulin. Plasma, serum, or urine may be used. Insulin is valuable in diagnosing insulinoma and evaluating types of diabetes. Hypoglycemic patients are among those who benefit from a technique of determining insulin levels. The test is helpful in diagnosing these persons, and can also be a diagnostic laboratory test for insulinoma. In this latter test, insulin levels are measured after each of a tolbutamide and a glucose administration.

Growth hormone. This test aids in determining causes of abnormal growth as well as evaluating pituitary function. Assay for growth hormone is ordinarily performed on children to determine if the lack of hormone is the cause of dwarfism or an overabundance is resulting in giantism.

Thyroxin (T-4). Plasma, serum, or urine may be used. Thyroxin is the major source of organic iodine, and is measured to evaluate thyroid function. The RIA method measures T-4 bound to thyroid-binding globulin (TBG).

Triiodothyronine (T-3). Plasma, serum, or urine may be used. T-3 helps determine abnormal thyroid function. It is believed to be the most biologically active of the thyroid hormones. Of all the radioimmunoassay tests currently requested, the vast majority are for T-3's.

Thyroid-stimulating hormone (TSH). This aids in evaluating both thyroid and pituitary function. TSH, whose function is to stimulate the thyroid to release its hormone, is produced by the pituitary. Through a TSH analysis, it can be determined whether lowered thyroid activity is due to a defect in that gland, or whether the problem lies in the pituitary.

Adrenocorticotropin. This helps in evaluating pituitary/adrenal function. The adrenal gland is dependent upon ACTH for secretion of the corticosteroids and androgenic hormones, but not for aldosterone secretion.

Cortisol. This aids in evaluating adrenal function. It is one of the hormones that regulate gluconeogenesis. Overproduction causes the physical condition noted in Cushing's syndrome. Cortisol, as one of the glucocorticoids, is currently commonly measured in the urine as part of the 17-OH corticosteroids. Stress, such as surgery, burns, pancreatitis, and eclampsia, will raise the plasma level. Low or low normals are found in Addison's disease and in anterior pituitary hypofunction.

Follicle-stimulating hormone. FSH helps to evaluate the pituitary/gonadal interaction. FSH measurement is of value in differentiating numerous endocrine dysfunctions, and particularly in distinguishing between primary and secondary gonadal failure.

Luteinizing hormone (LH). LH aids in diagnosing pituitary/gonadal problems, and in evaluating fertility problems in females. With FSH, LH acts to bring maturation of the follicle, and is also necessary for development of a functioning corpus luteum and for production of progesterone. LH analysis can verify occurrence of ovulation.

Testosterone. This aids in evaluating both testicular and adrenal function. In males, low levels of testosterone have been found in hypopituitarism, hypogonadism, and hepatic cirrhosis. In women, polycystic ovary and idiopathic hirsutism increase testosterone values. In addition, several types of tumors secrete testosterone.

Estrogens. These help evaluate fertility and ovarian function. The most commonly measured estrogens are estradiol, estrone, and estrol. The significance of these determinations have been previously discussed.

Progesterone. This helps evaluate fertility and to measure placental function during pregnancy. Progesterone is not found in the urine. Measurement of pregnanediol in urine is conducted for the same purpose as progesterone analysis in serum. Pregnanediol is a product of progesterone.

Chorionic somatotrophin (placental lactogen). This monitors progress of pregnancy. HCS, produced by the placenta, is involved in supplying glucose to the fetus. Working in conjunction with insulin, HCS mobilizes stored fat and leaves more glucose free for fetal use. HSC/insulin action also helps maintain a high level of amino acids in the blood, providing enough protein for the fetus during its rapid growth. A fall in HCS during pregnancy indicates placental dysfunction and fetal danger or distress.

Vitamin B-12. This aids in diagnosing pernicious anemia and malabsorption disorders. Serum levels of vitamin B-12, also known as extrinsic factor, reflect nutrition when liver disease and chronic leukemia have been ruled out.

Folate. This helps evaluate the cause of anemia. Folate, necessary for hematopoiesis, is found to be deficient in many infants with infection. Deficiency leads to megaloblastic anemia. Both B-12 and folate analyses must usually be performed to determine which is deficient.

Immunoglobulins. These aid in diagnosis of disorders of the immune state. Quantitation of a specific immunoglobulin is often needed after the proteins have been visualized by immunodiffusion. There is a wide range of gammopathies, including deficient levels and appearance of M proteins. Immunoglobulin E has been associated with allergic disease and is currently under intensive research study.

Carcinoembryonic antigen (CEA). This helps as a diagnostic aid for patients with signs, symptoms, and clinical history compatible with cancer. CEA, so called because of its presence in fetal tissue, is absent in normal adult humans except in cases of cancer of the large intestine. In some cases, the antigen can be detected before clinical evidence of the disease.

Hepatitis-associated antigen. This may help diagnose hepatitis and determine potential carriers. Of major interest to blood banks, this test spots hepatitis carriers, helps determine presence of the disease, and may also help the physician follow the course of infection.

Morphine. This helps to determine drug ingestion. Morphine, the metabolite of heroin, is the most commonly measured substance to determine addiction to heroin. The recent development of a rapid means of assaying morphine by radioimmunoassay may turn this procedure into a method of choice. Research is being done on developing radioimmunoassay procedures to test other drugs of abuse.

The routine laboratory should avoid RIA procedures that are cumbersome and involve iodination of substrates. However, most of the procedures are now relatively simple and have graduated from the realm of research.

Exercises (422):

Match the following by placing the letter of the column B item beside the number of the column A item or items that most nearly describe it. Each element in column B may be used once, more than once, or not at all.

Column A

___ 1. Involves the specific binding of an antibody to an antigen which is measured.

___ 2. Produced in various designated animals by the injections, at repeated intervals, of a substance foreign to the animal.

___ 3. Method of choice for measuring hormones with a low circulatory concentration in blood.

___ 4. Incubation of constant quantities of labeled antigen and antibodies with a series of known standard amounts of unlabeled antigen.

___ 5. Determination of the bound radioactivity. Bound, free, or both radioactivities may be counted.

___ 6. Plasma (EDTA) required helps diagnose and evaluate renal hypertension.

___ 7. Requires a 24-hour urine sample. Its increase can be caused by salt depletion, cardiac failure, cirrhosis, nephrosis, and pregnancy.

___ 8. Levels are measured after each of a tolbutamide and a glucose tolerance.

___ 9. Believed to be the most biologically active of the thyroid hormones.

___ 10. Stress, such as surgery, burns, pancreatitis, and eclampsia, will raise the plasma level.

___ 11. In males, low levels have been found in hypopituitarism, hypogonadism, and hepatic cirrhosis; and secreted by several types of tumors.

___ 12. Evaluate fertility and ovarian function.

___ 13. Helps evaluate fertility and measure placental function during pregnancy.

___ 14. Aids in diagnosing pernicious anemia and malabsorption disorders.

___ 15. Helps to evaluate the cause of anemia; necessary for hematopoiesis. Found to be deficient in infants with many infections.

___ 16. Helps as a diagnostic aid for patients with signs, symptoms, and clinical history compatible with cancer.

___ 17. Helps diagnose hepatitis and determine potential carriers.

___ 18. The metabolite of heroine; the most commonly measured substance to determine addiction to heroin.

Column B

a. Radioimmunoassay (RIA).
b. Competitive protein binding (CPB).
c. Antibodies used in radioimmunoassay.
d. First step in RIA procedure.
e. Third step in RIA procedure.
f. Second step in RIA procedure.
g. Digoxin.
h. Morphine.
i. Hepatitis-associated antigen.
j. Carcinoembryonic antigen (CEA).
k. Progesterone.
l. Folate.
m. Immunoglobulins.
n. Vitamin B-12.
o. FSH.
p. Testosterone.
q. Thyroxin (T_4).
r. Triiodothyronine (T_3).
s. Cortisol.
t. Aldosterone.
u. Renin (Angiotensin I).
v. Insulin.
w. Estrogens.

423. Point out procedures for determination of urinary metabolites in terms of production, abnormal production, methods, and reasoning behind methods.

Serotonin and 5-Hydroxyindolacetic Acid (HIAA). Serotonin is produced by the argentaffin cells of the intestine from tryptophan and is excreted as the metabolite of 5-hydroxyindolacetic acid. Excessive amounts of serotonin are produced in carcinoid tumors by the argentaffin cells.

Initially, tryptophan is oxidized and then decarboxylated to form serotonin. The breakdown occurs in oxidative deamination by the enzyme amino oxidase. Serotonin, produced by the argentaffin cells of the intestine, is normally localized in the gastrointestinal mucosa. Small amounts are detected in the spleen due to platelet disintegration and some in the brain. In patients with carcinoid tumors that have metastasized to the liver and the nodes, more than half of the daily tryptophan is diverted by the tumor into the serotonin pathway. It is presently recognized that the estimation of urinary 5-HIAA, which appears in large amounts in these cases, is the best diagnostic method. In cases of argentaffinoma, increased amounts of 5-HIAA are found in the urine.

Methods of estimation are based on the photometric measurement or visual comparison of the color complex formed by 5-HIAA with nitrous acid and 1-nitroso-2-naphthol. The qualitative test is not specific for 5-HIAA and measures 5-hydroxyindoles. Thus, a positive test should be followed by a quantitative determination. A normal urine might show a slight yellow color. SA positive test shows a purple color.

In the quantitative test, acidified urine is treated with 2, 4 dinitrophenylhydrazine for removal of interfering keto acids and extraction with chloroform to remove the indolacetic acid. The 5-HIAA is then extracted into ether, then reextracted into small volumes of phosphate buffer, and reacted with nitrous acid and 1-nitroso-2-naphthol. The normal range of excretions is 2-9 mg/24 hours. In the presence of a carcinoid or islet type tumor, the level rises to 25-1000 mg/24 hours. Prior to collection of the 24-hour urine specimen, 10 ml of HCL is added as a preservative and to maintain an acid pH during collection. Metabolites of chloropromazine interfere with color formation. Patients should abstain from this drug while urine specimens are collected. Foods known to contain large amounts of serotonin may lead to elevated results. Such foods as egg plant, avocados, bananas, pineapple, and plums should be avoided prior to and during collection of specimen.

Catecholamines and Vanillylmandelic Acid. The beta catecholamines include two active components which have been isolated from the adrenal medulla. These two compounds are epinephrine (adrenalin) and norepinephrine (noradrenalin). Chemically the catecholamines have properties which are similar to alcohols, phenols, and amines. Norepinephrine stimulates the hypothalmus and the anterior pituitary. Epinephrine is similar in its pharmacologic and chemical properties to norepinephrine except that epinephrine possesses an n-methyl group. Norepinephrine does not produce the anxiety and apprehension caused by epinephrine. Epinephrine is used therapeutically as a vasoconstrictor, as a cardiac stimulant, to induce uterine contractions, and to relax bronchioles. The synthesis of epinephrine and norepinephrine in the body begins with two amino acids, phenylalanine and tyrosine. Epinephrine is produced by the methylation of norepinephrine. Catecholamines are metabolized principally via methylation to produce 3-methoxy-4-hydroxy derivatives. Upon oxidation, the methoxyamines produce corresponding acids. The major acid produced is 3-methoxy-4-hydroxy mandelic acid—also known as vanillylmandelic acid (VMA).

Catecholamine determination. Several different procedures have been used for the determination of catecholamines. They may be classified into four groups; biologic, pharmacologic, chemical-colorimetric, and chemical-fluoremetric.

a. Biologic methods use strips of rabbit aorta and measure the contractile responses to urine containing epinephrine and norepinephrine.

b. Pharmacologic methods require the measurement of blood pressure responses to epinephrine and norepinephrine blocking agents such as histamine and other such substances.

c. The chemical-colorimetric techniques have proved to be less sensitive and nonspecific when applied to urine.

d. Physicochemical procedures such as paper chromatography are acceptable methods. The chromatograms must be examined under ultraviolet light.

In one method, catecholamines are absorbed from alkaline urine into activated alumina, eluted with sulfuric acid, and reacted with reagents that produce a fluorescent derivation. The fluorescence is compared with that of standards processed in a similar manner, using a photofluorometer for quantitation.

A 24-hour urine specimen is used with 1 ml of concentrated H_2SO_4 added. The patient is required to abstain from any drug ingestion for 1 week prior to specimen collection. In the screening for pheochromocytoma, a 24-hour excretion of up to 100 μg total free catecholamines is considered normal. Random sample: up to 18 μg/100 ml urine. Results indicating 100-200 μg/24 hours are considered borderline and above 200 μg are suggestive of disease. Some drugs that may interfere with the chemical reaction of the tests are caffeine, aminophylline, and ethanol. It is thus advisable to limit intake of these drugs for 1 week before collection.

Vanillylmandelic acid (VMA) determination. Urinary excretion of VMA is highly elevated in the urine of patients with pheochromocytoma and related tumors. Since the concentration of VMA in urine is much greater and methods of determination are simpler and adaptable to most laboratories, it is preferred rather than the more intricate determination of catecholamines. There are two basic methods in general use for the measurement of VMA in urine.

In the first method, urine is treated with activated magnesium silicate to remove most urinary chromogens. The vanillylmandelic acid is then extracted, along with other urinary phenolic acids, into ethyl acetate, subsequently reextracted into alkaline solution with potassium carbonate (K_2CO_3), and a color is developed with p-nitroaniline and reextracted in butonal. The absorbance of the butanol layer is measured at wavelengths of 540 and 450 nm.

In the second method, VMA is oxidized to vanillin, which is then extracted and measured directly at 360 nm or condensed with indole-phosphoric acid reagent. Methods involving the direct measurement of vanillin require a spectrophotometer sensitive in the near ultraviolet, and those involving the reaction of vanillin with indole require an extra chemical reaction. Normal values by the extraction procedure range from 0.7 to 6.8 mg/24 hours, and those by the diazotized p-nitroanaline range from 2-14 mg/24 hours.

The 24-hour urine specimen must be preserved with 10 ml of concentrated HCl added to the container prior to collection.

Regardless of method, urine *must* be collected and maintained at a pH of less than 3.0 by the addition of acid to the collection container. Remember that appropriate warning must be given to the patient concerning concentrated acid. Certain medications, coffee, and some foods, particularly those which contain vanilla, are restricted for a period of 48 hours before collecting the specimen. The antibiotic nalidixic acid (NegGram) increases the apparent VMA value by producing an interfering drug metabolite.

The assay of VMA is performed to aid in the diagnosis of chromaffin cell tumors (so called because these cells stain readily with chromium salts), especially extramedullary chromaffin tumors. Slight elevation of VMA may be observed in cases of malignant hypertension, but this is usually not to the extent observed with a tumorous condition.

Exercises (423):

1. In what condition are excessive amounts of serotonin produced?

2. How is serotonin formed?

3. What cells produce serotonin?

4. Why is the estimation of the 5-HIAA considered the best diagnostic method for argentaffinoma?

5. What reagents cause the color complex in the method given for 5-HIAA determinations?

6. In the quantitative procedure, what reagents are used to remove interfering keto acids and indoloacetic acid?

7. Which foods containing serotonin can lead to elevated results of the 5-HIAA?

8. What reagent is added to the specimen container prior to collection? Why?

In exercises 9 through 18 indicate whether the sentence is true (T) or false (F). If you indicate "false," explain your answer.

T F 9. The two beta catecholamines isolated from the adrenal medulla are epinephrine and adrenalin.

T F 10. VMA is a urinary metabolite of the catecholamine via methylation.

T F 11. Chemical-colorimetric techniques for catecholamine determination have proved to be more sensitive and specific when applied to urine.

T F 12. Caffeine, ethanol, and aminophyline will all interfere with the chemical reaction in the catecholamine determinations.

T F 13. Absorbed catecholamines are eluted with sulfuric acid and reacted with a fluorescent derivative.

T F 14. In one method for VMA, urine is treated with magnesium silicate to remove the acid.

T F 15. After reextraction with potassium carbonate (K_2CO_3), the solution is diazotized with p-nitroaniline for color development and reextracted in butonal.

T F 16. Methods involving the direct measurement of vanillin require a spectrophotometer sensitive in the visible range.

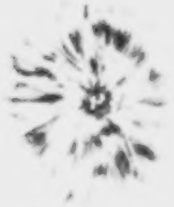


Figure 3-6. Section of urinary calculus showing concentric circles of salt deposition.

T F 17. The normal values given for the extraction procedure are 2-14 mg/24 hours.

T F 18. The antibiotic neomycin increases the VMA value by producing interfering drug metabolites.

3-3. Urinary Calculi

Analysis of calculi or "stones" from the urinary tract or gallbladder are usually performed in referral laboratories in the Air Force. The qualitative chemical tests are easy to perform, but require a number of reagents and manipulations. The frequency of calculus analysis is not sufficient to warrant providing the test in smaller medical units. However, technicians in referring laboratories should be aware of the general technique to better understand their responsibilities.

424. Tell the body sites where calculi may occur, reasons for identification of urinary calculi, and types of stones commonly found; indicate whether given statements concerning analysis of urinary calculi are true or false.

Significance of Calculi. Calculi may occur in the urinary tract, gallbladder, prostate, and occasionally in other sites such as the tonsils and salivary glands. Urinary tract calculi may be found in the renal pelvis, ureter, bladder, or urethra. Calculi in the renal pelvis or ureter are of particular clinical significance because they are frequently associated with serious renal disease, or they may be the causative agents of renal colic. Although the exact cause of renal calculi formation is unknown, it is hoped that the composition of the calculi will aid the physician in preventing future stone formation and in diagnosing the underlying disease.

The incidence of urinary calculi in Americans is high in middle-aged and elderly individuals. Their occurrence in any group depends upon diet, immobilization (as in paralysis), a limited urine pH range, or lowered fluid intake and the presence of various salts which form the calculi. Calcium oxalate stones and mixed stones of calcium oxalate and the carbonate or phosphate salt are most commonly found. Next, magnesium ammonium phosphate stones are most frequent, while uric acid appears in about 5 percent of cases. Other compounds mentioned below are infrequently present. The nidus or initial substance is often organic in nature (epithelium, fibrin, bacteria). This nucleus can be seen in cross sections of the calculus. Concentric circles of salt deposition may also be seen macroscopically. The nidus and concentric circles of deposition are pictured in figure

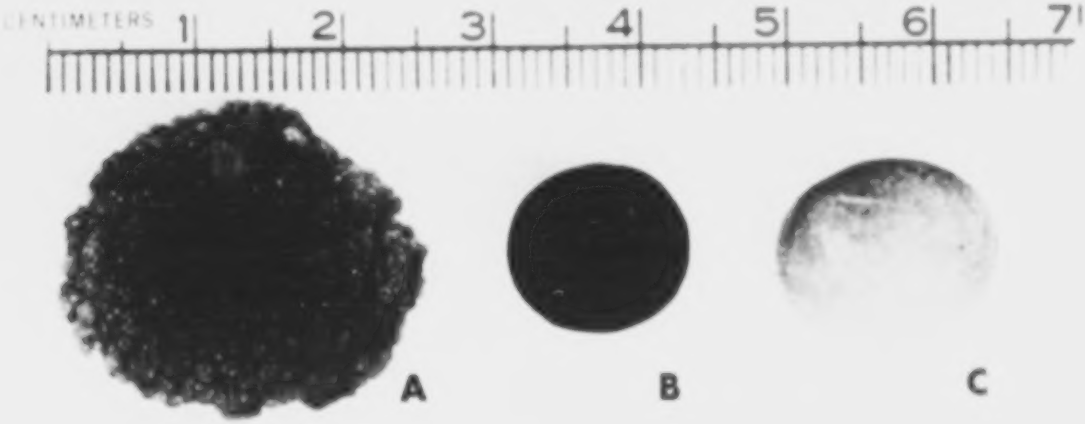


Figure 3-7. Relative size and physical characteristics of gall (a and b) and urinary calculus (c) stones.

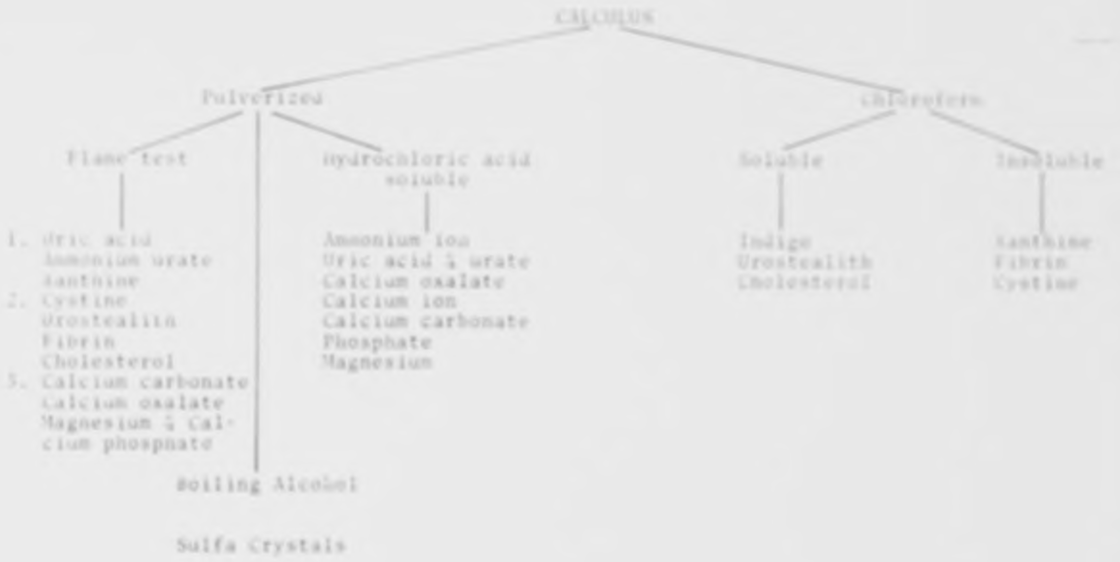

CALCULUS
Pulverized
Chloroform
Flame test
Hydrochloric acid soluble
Soluble
Insoluble
1. Uric acid
Ammonium urate
Xanthine
2. Cystine
Urostealith
Fibrin
Cholesterol
3. Calcium carbonate
Calcium oxalate
Magnesium & Calcium phosphate
Ammonium ion
Uric acid & urate
Calcium oxalate
Calcium ion
Calcium carbonate
Phosphate
Magnesium
Indigo
Urostealith
Cholesterol
Xanthine
Fibrin
Cystine
Boiling Alcohol
Sulfa Crystals

Figure 3-8. Outline for calculus analysis.

3-6. Under optimal conditions of salt concentration, pH, etc., successive deposits of salts are incorporated into a calculus around the nucleus material. Calculi vary in size from barely visible to as large as the calyx of the kidney. Smaller stones are passed in the urine, but larger stones must be removed surgically. You can see the relative size and physical characteristics of two gallstones and one urinary calculus in figure 3-7.

Methods of Calculi Analysis. The methods for analysis of calculi are explained below.

Thin section technique. In this technique, sections of the calculi are ground and polished. The structure may be examined by reflected light or by polarized light if the sections are thin enough to transmit light. This technique is used chiefly for photographic reproductions but is limited in adequate identification of components.

Infrared absorption spectroscopy. In this method, a powdered sample is placed in a bromide (KBr) disk. This allows precise qualitative identification of both single components and mixtures in some semiquantitative estimations.

X-ray diffraction. A powdered sample is used in this technique and requires 0.1 mg or less, offering a precise means of identification.

Optical method. In this method, the calculus is broken, dissected, and individual crystals identified by determinations of the refractive index. This is done under a chemical microscope by immersion in a series of fluids of known refractive index. It has been claimed that opaque impurities render this technique useless.

Chemical analysis. The qualitative chemical analysis has been, and still is, by far the most widely used technique. Many schemata for chemical analysis are available in literature. In the chemical analysis of calculi, the stone is divided and one half pulverized. A schema is presented in figure 3-8. Part of the pulverized material is subjected to a screening flame test which identifies the burning characteristics of certain groups of compounds. Then more specific qualitative tests (for example, nesslerization for ammonium ion, phosphotungstic acid—uric acid) identify definite chemical components. If sulfa drugs are present, they are dissolved with boiling alcohol, which is evaporated to dryness, and the crystals identified microscopically. Chloroform soluble portions of the remaining stone are tested for indigo, urostealith, and cholesterol; then any insoluble residue is examined for xanthine, fibrin, and cystine.

As indicated earlier, there are many schemes available for identification, and the given schema represents an isolated example. However, the inadequacy of the routine laboratory to perform time-consuming and detailed chemical analysis of calcula suggests that they be referred to laboratories specializing in the above-mentioned methods. The use of chloroform, however, in the routine laboratory where conditions are not controlled during its use presents an occupational hazard and should be discontinued.

Exercises (424):

1. In addition to the urinary tract, from what other body sources may calculi be obtained?

2. Give two reasons for the identification of urinary calculi.

3. What types of stones are most commonly found?

In questions 4 through 10, indicate whether the statement is true (T) or false (F). If you indicate "false," explain your answer.

T F 4. In the thin section technique, the calculi are ground and polished and examined under oil immersion.

T F 5. In the infrared absorption spectroscopy, the powdered sample is incorporated in a KBr disk.

T F 6. In the optical method, the calculus is broken, dissected, and the refractive index of the individual crystals determined.

T F 7. The chemical analysis is the least used technique.

T F 8. In the chemical analysis, part of the pulverized material is subjected to a screening flame test which identifies the burning characteristics of certain groups of compound.

T F 9. The X-ray diffraction technique is affected by opaque X-ray impurities.

T F 10. The use of chloroform in the routine laboratory presents an occupational hazard and should be discontinued.

Toxicology

THE NEED FOR drug testing increases as a result of the growing problem of overdose and the rapidly increasing number of addicts identified throughout the U.S. Air Force members are not immune to these conditions. The use and abuse of drugs have been discussed in newspapers, magazines, pamphlets of all kinds—as well as on radio and TV.

Since drug abuse has a particularly serious consequence for the Armed Forces, an intense effort is made to provide faster, easier, and cheaper means of testing for drugs of abuse.

On the other front of growing threats is the increase in the number of potent therapeutic drugs, newer insecticides, pesticides, and industrial compounds of a toxic nature which have all added to the lists of potential poisons. The study of poisons and their effects on the human body is called toxicology.

In order to obtain the correct diagnosis, the determination of the presence or absence of toxic compounds in body fluids may be necessary. Just as important is the knowledge of the therapeutic levels of some drugs in the blood or other body fluids, which may be helpful in prescribing safe and proper therapy.

The modern, well-equipped clinical toxicology laboratory should include thin-layer chromatographic materials, ultraviolet spectrophotometer, gas liquid chromatography, atomic absorption spectrometer, and radiation counter, as well as related equipment items, materials, space, and personnel.

Procedures common to the capabilities of most Air Force laboratories will be discussed in this section. Only a few toxicology studies will be discussed in detail in this chapter, namely those having a very general application and usefulness. Familiarization with areas discussed here will prove of value at a time when implementation of seldom-used emergency procedures can prevent confusion and inaccuracy. However infrequently a procedure is performed, the technician should have a high degree of confidence and proficiency.

4-1. Routine Toxicology in Air Force Laboratories

Aspirin, alcohol, and carbon monoxide are so prevalent that every hospital laboratory should have chemistry procedures available to test for them. Toxicology tests provide the physician with information useful in one of three situations. First, it may be clinically desirable to establish therapeutic levels of certain drugs in the blood. Hence, laboratory data must be accurate and timely. Second, diagnosis may depend upon ruling out the effect of drugs. For example, physiological or behavior changes due to a brain tumor, encephalitis, etc., can sometimes be confused with the effect of drugs unless the latter is ruled out or confirmed by the laboratory. Third, the possibility of accidental or intentional overdose of toxic substances may require treatment. Children frequently suffer accidental poisoning from drugs (or other chemicals) within their reach, especially aspirin. Also of particular interest to nearly all clinical laboratories is the blood alcohol, which will be discussed at some length in this section.

425. Distinguish between sobriety determination and blood alcohol test, and cite controlled procedures in processing specimens.

Medico-Legal Aspects of Blood Alcohol Tests. One of the characteristically vague areas of the clinical laboratory is that which is concerned with the legal (forensic) aspects of laboratory procedures. Of the medico-legal problems which could involve the laboratory, proof of intoxication is one of the most likely. It is not uncommon for a commander to refer someone to the hospital for a *sobriety* evaluation. The term "sobriety" refers to a clinical opinion which only a physician should render. A blood alcohol test result is usually part of the contributing evidence in a sobriety evaluation; yet, in reporting a blood alcohol test result, the technician is not stating whether the patient is or is not intoxicated. You should clearly understand the difference between a "sobriety" test and a "blood alcohol" test. The former implies clinical opinion which a laboratory technician is not professionally qualified to render.

When you receive a request for a blood alcohol determination, you must be careful to observe precautions which are more or less unique to a legal situation. Sometimes, blood alcohol studies do not have a special legal meaning. For example, an unconscious patient may appear to be intoxicated when he is in reality suffering from injury or disease. In this case, the medical officer may request a blood alcohol test for diagnostic purposes. If possible, to resolve any question about the legal implications, the technician should communicate directly with the physician. If the case involves the rights of the patient as a citizen rather than purely diagnostic factors, certain considerations become significant. These considerations are discussed in the following paragraphs.

It may prove important that the request to perform the test is valid. Hospital policy makes it clear in most cases who may order laboratory procedures and how this is done. Ordinarily, a request does not have to be in writing. At the time a test is ordered, a medical officer will probably state whether or not the case has legal significance. If the results of the test are to be considered for admission as evidence in a court, it is vital to protect the rights of the individual. Rights include those guaranteed by the fifth amendment to the Constitution and reiterated in Article 31 of the Uniform Code of Military Justice. These rights must not be disregarded.

How much responsibility do you have in protecting the patient's rights? Should you draw the blood specimen and perform the test for which you have an order; or should you be concerned with legal responsibilities? Unfortunately, there is no simple answer to this question which can be set forth in a career development course. A technician is expected to follow all existing policies and directives as well as exercise good judgment commensurate with his training and experience. Law experts recognize that circumstances vary with each case, and this is, in fact, a reason for the time-consuming and expensive proceedings of military and civilian courts. The underlying reason, of course, is to protect both society and the rights of the individual. A technician who follows local policy and does not attempt decisions for which he has no authority is on safe ground. In some medical facilities it is policy for the person who draws the specimen to inform the patient of his rights under Article 31 of the UCMJ. Failure to do so would place laboratory evidence in a highly questionable light with regard to admissibility in court.

A laboratory employee should never draw blood against the patient's will unless he is specifically ordered to do so by competent authority. Then he should be aware of the patient's possible legal reaction to his act and be prepared to prove that drawing the specimen was legally acceptable and right. Insofar as possible, try to anticipate the various possibilities and find out in advance what is required. Those in a nonsupervisory capacity should be particularly careful to keep their supervisors informed of circumstances surrounding medical-legal situations. Supervisors will undoubtedly consult the Staff Judge Advocate in all cases of doubt.

Processing Blood Alcohol Specimens. When you draw a specimen, do not use alcohol as a skin cleansing agent (remember that all tinctures are alcoholic solutions by definition). If analysis is based on a reduction procedure, other reducing substances like acetone should not be used either. This is not so much a matter of probable contamination as one of technical doubt which may be raised in evaluating the results. You may use aqueous iodine or aqueous merthiolate before venipuncture.

Security is another consideration. The specimen must be labeled and secured so that it cannot be tampered with or confused with another specimen. A locked box which is kept in the refrigerator is useful for this purpose, provided access to it is controlled. It is usual procedure to maintain a *chain of custody receipt*, which is a log showing how and by whom the specimen is handled from the time it is drawn until the test is completed. If the specimen is mailed, it should be sent by registered mail.

All readings and calculations must be checked and verified according to the existing policies of the laboratory concerned. Both a standard and at least one control must be run with a conscious effort to avoid cross-contamination. Records of the procedure should be kept to provide a concise and complete account of the analysis. You may need to refer to these records at a later date if you are called upon to testify in court.

Reports of a blood alcohol determination are to be kept within proper channels. Results are normally given only to a concerned medical officer or to someone designated by the commander. Reports of a blood alcohol test are *never* released out of medical channels by laboratory personnel without specific authorization to do so. Careless handling of a report may result in considerable difficulty and compromise. Questions concerning disposition of reports are referred to the hospital registrar by the laboratory supervisor. The results should be clearly designated as mg/ml or mg-%, properly dated, and signed. The time lapse between drawing a specimen and completing the test may in some instances affect legal acceptability of the results. We will discuss specimen stability later in this section. Keep in mind that any official report is a potential item of evidence in a court of law. Toxicology studies, and particularly blood alcohol tests, are the most likely items for legal consideration.

Exercises (425):

1. Explain the difference between a sobriety determination and a blood alcohol test.

2. If you are asked to inform an individual of his rights to avoid self-incrimination before you draw a blood alcohol specimen, where would you find a concise statement of these rights?

3. If your analysis of blood alcohol is based on a reduction procedure, could you use tincture of merthiolate to prepare the site before drawing the blood? Why or why not?

4. What is a chain of custody receipt? Why is it important?

5. How should a blood alcohol specimen be mailed?

6. To whom may blood alcohol reports be given?

7. When would it be permissible to discuss the blood alcohol results with a neighbor who is a security policeman?

426. Indicate whether given statements correctly reflect the physiological effects of ethanol and methanol in terms of metabolic process at varying concentrations and conditions of intoxication.

Chemistry and Metabolism of Ethanol. Ethyl alcohol, also known as grain alcohol or ethanol, has the relatively simple chemical formula C_2H_5OH. It has a toxic effect on various tissues of the body, especially the liver, if it is ingested in significant quantities over a prolonged period. Excessive intake in a short time may result in death. Ethanol is present in alcoholic beverages in concentrations varying from as little as 3 percent in some beers to approximately 50 percent in whiskies. A 50-percent solution of alcohol is termed 100 proof. As suggested in the chapter on gastric analysis, alcohol can be absorbed in the stomach through the gastric mucosa as well as in the small intestine. The blood level of alcohol rises more rapidly if the stomach is empty when the alcohol is consumed. Alcohol is distributed throughout the body and can be detected in tissue and body fluids, including spinal fluid. Less than 10 percent of the alcohol ingested appears in the urine, as it is steadily oxidized in liver and kidney tissue to carbon dioxide and water.

The metabolic process of breaking down alcohol is as follows: First, C_2H_5OH is transformed into acetaldehyde, CH_3CHO, which is then oxidized. Blocking the chemical oxidation of acetaldehyde at this point can be accomplished with drugs like disulfiram ("Antabuse"). The resultant accumulation of acetaldehyde causes extreme nausea and may be effective as a means of discouraging alcoholic intake. Acetaldehyde is normally converted to acetic acid, CH_3COOH, also known as ethanoic acid. Acetic acid then enters a metabolic cycle, resulting in final oxidation to carbon dioxide and water with the release of a significant number of calories. In the process of the breakdown of alcohol, other organic substances may be formed in small amounts which are destructive to kidney and liver tissue. Alcohol is excreted in the urine as noted above, but not to any great extent. Most of the alcohol in the blood is reabsorbed in the kidney tubules. Some is exhaled in vapor from the lungs, a phenomenon which makes possible the so-called balloon test for analyzing alcohol level of breath. Breath analysis for alcohol content is based on the principle that the amount of alcohol in the expired air is representative of the amount in the blood at the time the sample is obtained. Breath analysis is employed frequently for law enforcement purposes.

Physiological Effects of Ethanol. It cannot be stated precisely at which level the person's physical or emotional condition changes. As a general rule, at a blood level of less than (0.05 percent) 50 mg-% (0.5 mg/ml) a person is asymptomatic. Coordination, physical control, and emotional stability deteriorate as the blood level rises; at a blood concentration of (0.150 percent) 150 mg-% (1.5 mg/ml) a person is considered "under the influence." Interpretation is, of course, a matter of clinical judgment, as noted previously. Based on actual observations, approximately 50 percent of the population will show clearly observable signs of intoxication at a blood level of 150 mg-%. At least 80 percent of all individuals with a blood level (0.200 percent) 200 mg-% appear to be intoxicated as the term is normally used. That is, their conduct and physical coordination are significantly affected. At blood levels of (0.300 percent) 300 mg per 100 ml of blood, few drinkers are able to control themselves. There is onset of coma at approximately (0.400 percent) 400 mg-%, from which some do not recover. Blood levels above (0.500 percent) 500 mg-% are nearly always fatal.

According to the National Safety Council Committee on Tests for Intoxication, the term "under the influence of alcohol" should be used instead of "intoxication." *Any* individual is considered to be "under the influence" when the alcohol level in the blood reaches 1.5 mg per ml. The blood of the average individual who has not been drinking should not contain alcohol, and thus a lack of alcohol should be reflected. The effects of alcohol at levels less than those which are fatal are well known to most people. The overall effect is that of a depressant.

Prolonged use of alcohol results in liver damage, commonly described as alcoholic cirrhosis. There are many associated symptoms of physical deterioration in chronic alcoholics, including vitamin deficiencies. Inability of the liver to excrete porphyrin in the bile may result in abnormal excretion of urinary porphyrins. Lipids in the urine (lipuria) may also result from alcoholism. In acute alcoholism, there is a moderate rise in the serum amylase level, as well as an abnormal glucose tolerance curve. A typoxic condition known as histotoxic hypoxia may also result. This is characterized by lack of oxygen available to the tissues. Many symptoms of alcohol poisoning are similar to those resulting from other toxic substances such as lead and organic solvents. Ethyl alcohol is least toxic of the alcohols.

Methyl alcohol (methanol, wood alcohol), CH_3OH, is far more toxic than ethanol. Blindness and death may result from any significant intake of methyl alcohol. There is very little physical difference between ethanol and methanol, which frequently results in the mistaken use of methanol. The two can be distinguished chemically, a problem which could be presented to a clinical laboratory if there is some question of whether a sample is methyl or ethyl alcohol.

Exercises (426):

Indicate whether each of the following statements is true (T) or false (F) and correct those that are false.

T F 1. Ethyl alcohol is also known as wood alcohol and has a simple chemical formula C_2H_5OH.

T F 2. If a 50-percent solution of alcohol is termed 100 proof, a 50 proof whiskey is 75 percent.

T F 3. Alcohol is absorbed in the stomach through the gastric mucosa and small intestines.

T F 4. Alcohol is distributed throughout the body and can be detected in tissue and all other body fluids *except* CSF.

T F 5. Less than 10 percent of the alcohol ingested appears in the urine as it is steadily oxidized by the liver and kidney tissue to carbon dioxide and water.

T F 6. In the first step of the metabolic process, C_2H_5OH is transformed into acetic acid.

T F 7. Disulfiram (Antabuse) or similar drugs may be used to block the chemical oxidation of acetaldehyde.

T F 8. Acetaldehyde is normally converted to ethanoic acid.

T F 9. In the process of breakdown, other organic substances formed in small amounts are harmless to kidney and liver tissue.

T F 10. A person is considered "under the influence" at a blood concentration of 50 mg-% (0.5 mg/ml).

T F 11. Blood levels above 500 mg-% (5.0 mg/ml) are nearly always fatal.

T F 12. The overall effect of alcohol is that of a stimulant.

T F 13. Lipids in the urine may also result from alcoholism.

T F 14. Acute alcoholism will cause a rise in the serum amylase and abnormal glucose tolerance curve.

T F 15. Methyl alcohol is far less toxic than ethanol.

T F 16. Much physical difference exists between ethanol and methanol which discourages the mistaken use of methanol.

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427. Identify the methods and reagents for testing ethanol and indicate possible sources of error.

Laboratory Tests for Ethanol. Quantitation and detection of ethanol is achieved by three different methods: dichromate, enzymatic (alcohol dehydrogenase), and gas chromatographic methods.

Dichromate method. The techniques using this method generally are based on the following principle: alcohol is diffused from the specimen into a solution of potassium dichromate in a diffusion chamber. The hexavalent chromium of dichromate is reduced by alcohol to trivalent chromium, resulting in a change of color from yellow to green. The intensity of the color developed in the acid dichromate is proportional to the concentration of alcohol. Reduction is illustrated as follows:

$$2K_2Cr_2O_7 + 8H_2SO_4 + 3C_2H_5OH \rightarrow 2K_2SO_4 + 3CH_3COOH + 11H_2O + 2Cr_2(SO_4)_3$$

Potassium dichromate-sulfuric acid solution is known as Ansties reagent. Other procedures involve a micro diffusion analysis with the reduction of Ansties reagent. An example of this method is the use of the Conway diffusion microdiffusion chamber. It is simple and relatively rapid. However, there is no distinction between ethanol and other volatile reducing substances. Alcohol is by no means the only substance which will reduce Ansties reagent. The presence of medications, ketone bodies, and lactic acid also reduces it. Many other volatile compounds such as methyl alcohol, isopropyl alcohol, ethyl ether, chloroform, and formaldehyde also reduce potassium dichromate to chromic ion. Therefore, the procedure is not specific for ethanol.

Enzymatic methods. The alcohol dehydrogenase technique is relatively simple, accurate, and specific. The other primary and secondary aliphatic alcohols do not produce false positives to adversely influence test results. It is based on the following reaction:

$$C_2H_5OH + NAD \rightleftharpoons CH_3CHO + NADH + H^+$$

This is an equilibrium reaction that can also go in the opposite direction; however, this reversal is prevented by the addition of semicarbazide which reacts with the acetaldehyde (CH_3CHO) as it is formed. Under proper conditions the absorbance reading at 340 nm of the NADH formed is proportional to ethanol concentration. Whole blood (no fluoride), serum, plasma, urine, or gastric washings may be used in this procedure. If the tube is tightly stoppered and refrigerated, the specimen is stable for several days. The reagents used in the technique described in AFM 160-49 are:

a. Buffered, pyrophosphate, pH 9.2.
b. Nicotinamide Adenine - dinucleotide - alcohol dehydrogenase.
c. Ethanol standard, 1.5 mg per ml.
d. 2 percent perchloric acid.

Since this is an enzymatic procedure, the reaction conditions must be strictly followed.

If post mortem examination of blood is performed, the analysis must be carried out as soon as possible, since falsely elevated results may be obtained due to putrefaction.

Gas chromatography. Gas chromatography is the method of choice for rapid, direct, and specific qualitative and quantitative analysis. This method is quite expensive compared to other methods and requires sophisticated instrumentation and specially qualified personnel. The procedure may be accomplished by head space analysis or by direct injection of blood onto the column. By gas chromatography other volatile substances beside ethanol may be identified and quantitated.

Exercises (427):

Match the following items in column B with the statements to which they are most closely related in column A by placing the letter of the column B item beside the number of the column A item. Each item in column B may be used once, more than once, or not at all.

Column A

___ 1. The overall method of choice for quantitative estimation of blood alcohol.
___ 2. Other volatile substances besides ethanol may be identified and quantitated by this method.
___ 3. May cause falsely elevated results in post mortem sample if not performed ASAP.
___ 4. A method of the Conway microdiffusion technique.
___ 5. Will reduce Ansties reagent.
___ 6. Method not significantly affected by the primary and secondary aliphatic alcohols.
___ 7. Under proper conditions the absorbance reading at 340 nm of the NADH formed is proportional to ethanol concentration.
___ 8. Use of sodium flouride as anticoagulant will affect the results of the test in this method.

Column B

a. Dichromate method.
b. Enzymatic method.
c. Gas chromatography.
d. Ansties reagent.
e. Potassium dichromate.
f. Putrefaction.
g. Ethyl ether, chloroform, formaldehyde.
h. Kerosene, chromic acid.
i. Isopropyl, ketone, and lactic acid.

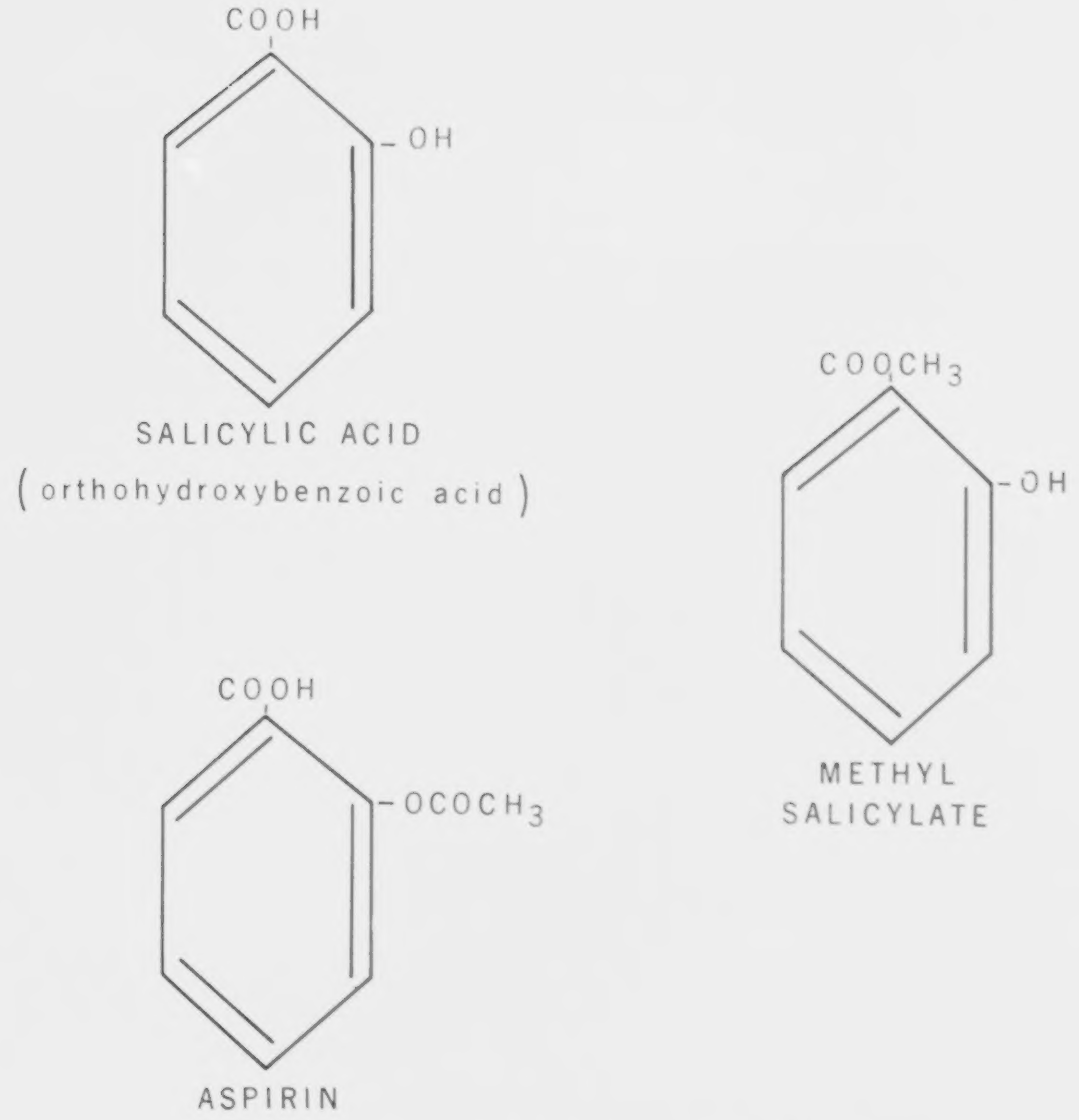

Figure 4-1. Structural formulas of salicylic acid, methyl salicylate, and aspirin.

428. State the physiological effects of salicylates and indicate the significance of the tests for blood salicylate level and methods of determination.

Salicylates. Aspirin, also known as acetyl salicylic acid, is the most frequently used of all medications. It is also the most common cause of poisoning in children between the ages of 1 and 4. Salicylic acid itself is too irritating to be used internally. Therefore, it is used therapeutically as derivatives of salicylic acid or organic acids. You can see that aspirin is an ester of acetic acid by examining figure 4-1. Compare the structure of salicylic acid with methyl salicylate (oil of winter green), well-known for its effects as a local irritant. Salicylates affect the central nervous system, specifically the hypothalamus, and by so doing control body temperature. The hypothalamus of the brain acts as a biological thermostat. Drugs, such as salicylates, which control this mechanism are called *antipyretics*. Salicylates also depress the central nervous system and thereby relieve pain. Hence, they are also termed *analgesics*. Salicylates have a significant stimulating effect on respiration, causing an increase in oxygen consumption and CO_2 production. Salicylates accomplish this by directly stimulating respiratory centers in the medulla.

If the intake of salicylates is excessive, the effects can be profound. Initially, there is respiratory alkalosis due to hyperventilation. This is characterized by an increase in the pCO_2 of the plasma. Bases are then lost in the urine, causing the blood pH to return to normal. This is only a stage of the process and is referred to as "compensated respiratory alkalosis."

Changes in acid-base balance described thus far are experienced even at therapeutic levels of salicylates. Persons with blood levels above 50 mg-% usually show toxic symptoms. Toxic doses cause increased pCO_2 of the plasma and increased loss of bicarbonates, thus shifting to a condition of metabolic acidosis. Impaired renal function caused by salicylates through vasomotor depression permits the accumulation of organic acids. Their effect on pH is in addition to the existing respiratory acidosis which results from CO_2 accumulation.

In summarizing the physiological response to salicylates, it can be said that there are two opposing effects. They are respiratory alkalosis from increased respiration and respiratory acidosis from increased CO_2 production. Metabolic acidosis from impaired renal function and loss of bicarbonate contribute to the problem of respiratory acidosis. Various metabolic effects of the salicylates are recognized with respect to the following metabolic activities:

a. Oxidative phosphorylation. Salicylates inhibit adenosine triphosphate (ATP) dependent reaction.

b. Carbohydrate metabolism. Many factors function here, in some cases lowering blood sugar levels, and in others causing hyperglycemia.

c. Nitrogen balance. There is a decreased synthesis and an increased breakdown of amino acids, which result in a negative nitrogen balance.

d. Fat metabolism. Salicylates enhance the breakdown of tissue fatty acids and inhibit their synthesis.

e. Enzymes. Salicylates decrease the activity of a number of enzymes.

Besides the metabolic effects, salicylates affect endocrine functions, particularly the adrenal medulla, adrenal cortex, and the thyroid gland. Salicylates are usually used for analgesic or antipyresis, as previously suggested. Dangerously high blood levels lead to coma and death. Since the patient may appear only slightly affected during the first few hours following ingestion, laboratory studies may be important in diagnosis as well as in following the blood salicylate level.

Laboratory Test for Salicylates. In a more recent and improved laboratory procedure for salicylates, the test is rapid and a smaller amount of serum is required. The method is described in AFM 160-49. Serum is reacted with acidified ferric nitrate solution to produce a purple color. The color is directly proportional to the concentration of salicylic acid present plus metabolic products containing the salicylic group. The procedure is set up in small test tubes labeled "Test," "Standard," and "Blank": 0.1 ml of serum is added to the tube labeled "Test"; 0.1 ml of standard to the tube labeled "Standard"; 0.9 ml of distilled water is added to the test and the standard and 1.0 ml to the blank; 1.0 ml of Trinder reagent is added to all three tubes. The tubes are centrifuged at high speed for 10 minutes. The supernatant is poured into small cuvettes. The absorbances of the test and standard are measured at 540 nm with the blank set at zero. The Trinder reagent contains mercuric chloride, which precipitates the protein, and ferric nitrate to form the color that is directly read in the supernatant.

Calculation is indicated as:

$$\text{mg Salicylate per dl} = \frac{Au}{As} \times 20$$

Serum or plasma may be used. A procedure for urine requires a preliminary 1:5 dilution with water and is treated similarly to serum. Prepare a urine blank by adding 0.2 ml of diluted urine to 2.0 ml of reagent; then add 1.6 ml of H_2O and 0.2 ml of 85 percent phosphoric acid. The absorbance of the reference blank is measured and subtracted from the test. Salicylates are used in treatment of rheumatic and rheumatoid conditions. Blood levels of salicylic acid ranging from 36 to 40 mg per dl are maintained in therapy of rheumatic fever. Since this approaches toxic levels, this procedure is useful in controlling dosage. This procedure is also helpful in diagnosing cases of accidental salicylate poisoning.

In salicylate intoxication, metabolic acidosis may be present. This can result in the presence of ketone bodies (acetoacetic acid, diacetic acid) in the urine. These substances may give a positive color with the reagent and thus interfere. A few minutes before analysis, the urine may be boiled to eliminate these interfering substances. Phenothiazine drugs may also give an interfering color if large doses have been administered. However, the amount of these substances in the blood is rarely high in sufficient quantities to cause any significant interference.

Exercises (428):

1. Aspirin is described as an *antipyretic* and as an *analgesic*. What do these terms mean?

2. How are salicylates able to control body temperature?

3. Describe the chemical nature of aspirin.

4. Describe the effect of salicylates on blood pH.

5. List some metabolic activities of the body which are affected by salicylates.

6. How can salicylates be tested for in the laboratory?

7. What purpose does the mercuric nitrate serve in the Trinder reagent?

8. In salicylate intoxication or metabolic acidosis, what are possible interfering substances in urine for a salicylate level?

9. How can interfering substances be eliminated from the urine?

10. At what concentration of salicylates are toxic effects considered to be produced?

429. Indicate the procedure for determination of carbon monoxide in terms of physical properties, harm done to the human body, normal blood level, principles of procedure, and care of specimens.

Carbon Monoxide (CO). More deaths are caused by carbon monoxide poisoning than by any other substance except alcohol. Pure carbon monoxide is flammable and colorless. It is also odorless in concentrations below 70 percent in the pure form. Faulty stoves and furnaces and the internal combustion engine are the most common sources of accidental carbon monoxide poisoning. The possibility of carbon monoxide poisoning is always a factor of great importance in the investigation of aircraft accidents. It is inevitable that most Air Force medical laboratory technicians become involved in this problem at one time or another. Therefore, every technician must know at least the elementary aspects of carbon monoxide poisoning and how it is detected.

When it is inhaled, carbon monoxide combines with hemoglobin to form carboxyhemoglobin. This imparts a bright, cherry red color to the blood. Because hemoglobin has a much greater affinity for carbon monoxide than for oxygen, carbon monoxide replaces oxygen and thereby causes anoxia. It is possible to displace carbon monoxide which is attached to the hemoglobin molecule with oxygen; therefore, a person suffering from carbon monoxide poisoning may be revived if he is given oxygen before respiratory arrest and coma ensue.

Blood levels above 40 percent carbon monoxide are nearly always fatal. At blood levels less than 40 percent, symptoms may vary from a slight headache to vertigo, ringing in the ears, and severe head pain. There are usually no symptoms at blood levels less than 5 percent. Because of carbon monoxide in the atmosphere, 0 to 5 percent is considered normal. A heavy smoker's blood normally contains about 8 percent carbon monoxide. For this reason his blood provides an ideal high normal control on CO analyses. Children and individuals with a low hemoglobin are more susceptible to carbon monoxide poisoning than are healthy adults. Both the concentration of the gas in the atmosphere and the time of exposure are also factors to be considered in predicting the physiological response.

Determination of Carbon Monoxide in Blood. As stated earlier, the cherry red color of blood suggests carbon monoxide poisoning. The actual blood level may be determined by any one of several methods, the most reliable being gas chromatography. There are also various screening tests in use, the simplest being a dilution test for the detection of carboxyhemoglobin. In this procedure, the blood is diluted with water so that the solution appears a faint pink. A solution of 20 percent NaOH is then added and quickly mixed. If the blood contains less than 20 percent carboxyhemoglobin, the pink color rapidly disappears and the solution becomes yellow. Persistence of the pink color for more than a few seconds indicates carbon monoxide levels above 20 percent. This test is simple and specific for carbon monoxide. However, the time element is critical. A delay in color change from pink to yellow *after more than a few seconds* following addition of the NaOH is considered a *negative* test. A negative control should be run for contrast.

Another common screening procedure is the palladium chloride method. Carbon monoxide, acid-liberated from a blood sample, reacts with palladium chloride in a microdiffusion cell, causing the release of metallic palladium which forms a "mirror" on the surface of the reagent. The palladium procedure is outlined in detail in AFM 160-49. Methods using the spectrophotometer have also been devised.

A spectrophotometric method may be used for determining carbon monoxide which involves sodium hydrosulfite reduction of a blood sample that has been diluted with 0.4 percent ammonia. Oxyhemoglobin is completely reduced in the presence of sodium hydrosulfite, whereas carboxyhemoglobin is not reduced. The optical density quotient is determined from readings at two different wavelengths, and the percentage of carboxyhemoglobin is then read from a prepared curve.

Carbon monoxide for standardization may be used from a small gas cylinder, obtainable through Local Purchase, Supply. The generation of carbon monoxide by dropping concentrated sulfuric acid on formic acid in a flask is inconvenient, messy, and dangerous. Regardless of method, the specimen to be used is oxalated blood. It is usually stable until grossly hemolyzed or deteriorated. A few days' delay, as may be encountered in mailing the specimen, should not affect the results significantly. Confirmation of results by a reference laboratory may be desirable, especially after aircraft accidents and in medico-legal cases.

Exercises (429):

1. What are the physical properties of carbon monoxide?

2. Explain why carbon monoxide is harmful if inhaled.

3. List three factors that determine how harmful exposure to carbon monoxide may be.

4. What is the normal CO level of blood?

5. Describe the principle of the palladium procedure for CO determination.

6. What effect does sodium hydrosulfite have on oxyhemoglobin?

7. A physician requests that a blood specimen be mailed for a carbon monoxide test (your laboratory does not have a carbon monoxide procedure). How reliable would you consider the result after the specimen remains at room temperature in the postal system for 24 hours?

4-2. Special Toxicology Studies

The classification presented in this section is a very brief synopsis of the Gradwohl modification and by no means adequately covers the complex science of toxicology. Students who desire more detailed information may refer to a textbook of toxicology. Qualitative differentiation of toxic substances must not be oversimplified, especially if the analysis is performed on body tissue instead of on an isolated chemical. Interference and lack of specificity can easily lead to erroneous conclusions when toxicology tests are performed by inexperienced personnel.

430. Identify the basic principles and reagents involved in the detection and quantitation of volatile poisons.

Volatile Poisons. Toxicology tests other than alcohol are often requested for volatile substances. Since most laboratories are not equipped and staffed to perform detailed toxicological examinations, it will be necessary to refer the specimen to a consultant laboratory with such capabilities. The specimen should be shipped according to standard directives for specimen preparation and the proper chain of custody maintained. Some basic knowledge of the method of analysis of volatile substances may help you to understand the need for the type of procedures used and techniques for processing.

Acid-steam distillation of volatile poisons. It is sometimes necessary to prepare an acid distillate such as is needed for rapid spot tests. A portion of tissue (for example, 100 g of brain) is homogenized in a cold Waring blender. The homogenate is then transferred to a Florence flask together with a rinse of approximately 100 ml of distilled water. To this mixture 225 g of ammonium sulfate and 5 ml of concentrated sulfuric acid is added. The flask is connected to the steam distillation apparatus illustrated in figure 4-2. More sophisticated equipment is now available in many toxicology laboratories; however, the example illustrates the basic principle for acid-steam distillation of volatile poisons. A small portion of this distillate is used to test for volatile reducing substances, cyanides and sulfides, halogenated hydrocarbons, and phenols.

Volatile reducing substances. The purpose here is usually to rule out the presence of substances which might give a false positive test for ethanol. An acid dichromate solution, such as Anstie's reagent, will react with ethanol, methanol, acetaldehyde, and acetone. Procedures for differentiation can be found in a textbook of toxicology.

Cyanides and sulfides. Cyanides may be absorbed in alkali and converted to cyanogen chloride. The cyanogen chloride is reacted with methylphenyl pyrazalone to form a blue color. Sulfides may also be detected by conversion to bismuth sulfide or by the formation of a color complex with picric acid. If 5 ml

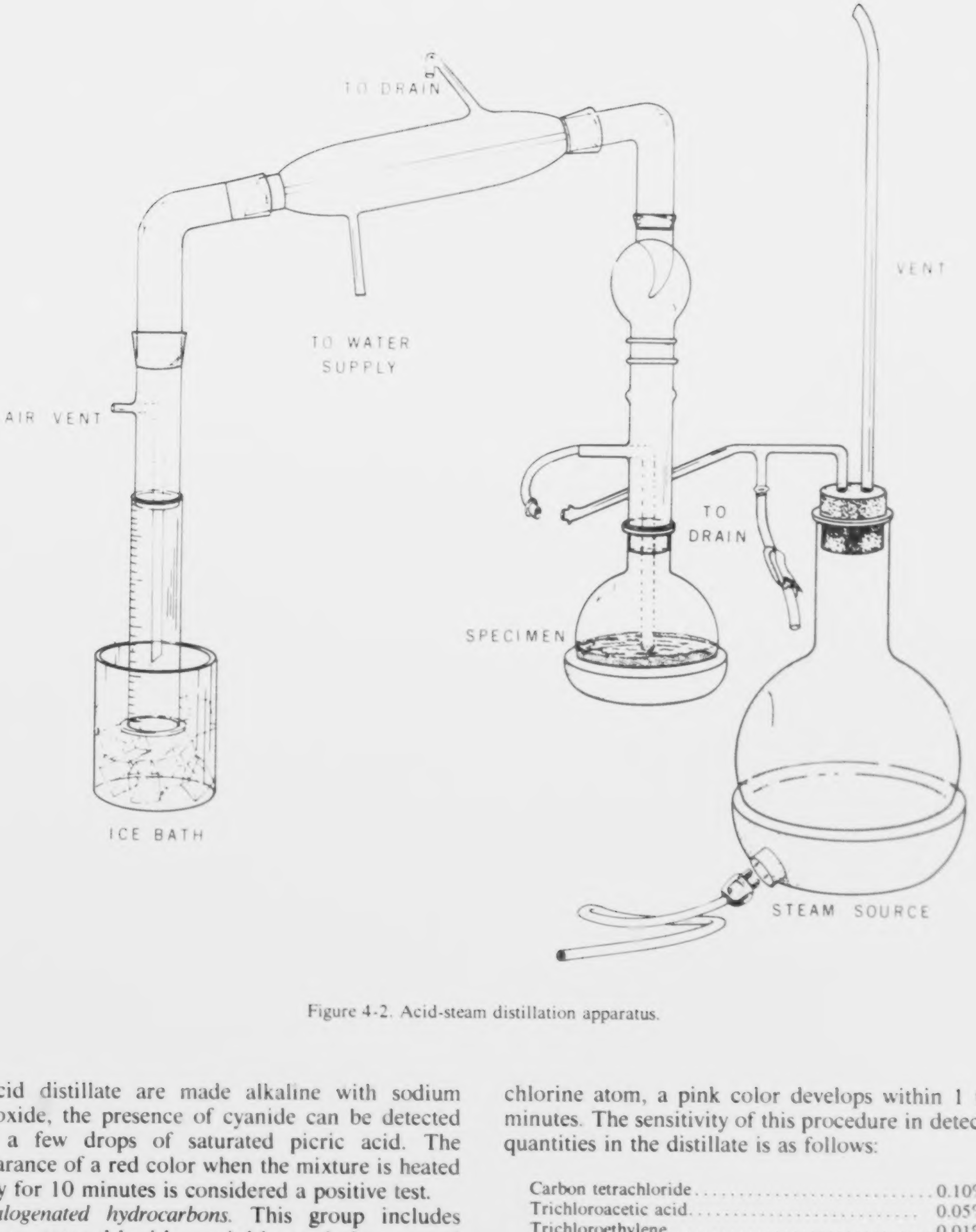

Figure 4-2. Acid-steam distillation apparatus.

of acid distillate are made alkaline with sodium hydroxide, the presence of cyanide can be detected with a few drops of saturated picric acid. The appearance of a red color when the mixture is heated gently for 10 minutes is considered a positive test.

Halogenated hydrocarbons. This group includes carbon tetrachloride, trichloroethylene, and chloroform. They may be separated by a microdiffusion principle. A popular means of detecting halogenated hydrocarbons involves the Fujiwara reaction. In this reaction 5 ml of acid distillate are added to 1 ml of redistilled pyridine. After the addition of 1 ml of 10 percent NaOH, the mixture is boiled for a few minutes. If chlorinated hydrocarbons are present which have more than one chlorine atom, a pink color develops within 1 to 3 minutes. The sensitivity of this procedure in detecting quantities in the distillate is as follows:

Carbon tetrachloride	0.10%
Trichloroacetic acid	0.05%
Trichloroethylene	0.01%
Chloral hydrate	0.005%
Chloroform	0.04%

Both positive and negative controls should be run.

Phenols. Chemicals with the characteristic phenol structure may be detected by the formation of insoluble tribromphenol. In one test, sufficient saturated bromine water is added to 5 ml of acid distillate. In the presence of phenols, a rather heavy yellow to white precipitate forms. The test detects as little as 0.002 percent phenol and 0.02 percent naphthol in the distillate. Other phenols are also detected, including cresol. Detection of members of each group in blood, urine, and tissue is excellent. The microdiffusion procedure has been shown to be rapid, convenient, and specific. Gas chromatography is sensitive, accurate, rapid, and simple for the separation, identification, and determination of volatile compounds. Temperature programming provides a faster and more versatile analysis.

Exercises (430):

Match the volatile substances in column B with the principles and methods of detection to which they closely relate in column A by placing the letter of the column B item beside the number of the column A item. Each column B item may be used once or more than once.

Column A

____ 1. Will develop a pink color within 1 to 3 minutes after boiling with 1 ml of 10 percent NaOH.

____ 2. Five ml of this acid distillate is added to 1 ml of redistilled pyridine.

____ 3. Detected by the formation of an insoluble tribromphenol.

____ 4. May be detected by the formation of a color complex with picric acid.

____ 5. Five ml of acid distillate are made alkaline with NaOH. A red color after the mixture is heated for 10 minutes is considered positive for this substance.

____ 6. Will reduce acid dichromate solution.

Column B

a. Acetaldehyde, formaldehyde acetone.
b. Cyanides.
c. Halogenated hydrocarbons.
d. Chlorinated hydrocarbons.
e. Phenols.
f. Chloroform.
g. Carbon tetrachloride.
h. Sulfides.

431. Indicate the procedures for drug screening in terms of specimens used for specific drugs, principles, and methods.

Drug Screening. Recent advances in the methods for rapid detection and identification of drugs of abuse and their metabolites in urine have been brought about by the growing problem of overdose and the rapidly increasing numbers of addicts. An intense effort is being made to provide faster, easier, and cheaper means of testing for drugs of abuse.

Many new systems are being manufactured to accommodate both the mass screening programs that are conducted throughout the Armed Forces, industries, and specialized medical laboratories. These drugs of interest include barbituates, amphetamines, the various tranquilizers, antihistamines, phenothiazines, diphenylhydantoin, opium alkaloids, and meperidine (Demerol).

The only popularly known drugs of abuse that cannot be readily detected in body fluids are hallucinogens, such as LSD, marijuana, and mescaline. Owing to the diversity of methods, we shall limit this discussion to the most common drugs of abuse and the methods of analysis.

Separation of the drug from urine is accomplished by either solvent extraction or by the use of nonionic exchange resin. The extract containing the drug is evaporated to small volume and the residue applied to thin-layer chromatographic sheets. After development with the solvent, the sheets are examined under ultraviolet light and then sprayed with a number of reagents in sequence to develop colors that differ for different types of drugs. The drugs are identified by the colors produced by their indicated values in comparison with known substances determined on the same sheet.

Barbiturates, Acetanilid, and Phenacetin. Barbiturates are widely used as prescription drugs to depress the central nervous system. After acid-ether extraction, barbiturate derivatives may be identified based on their reaction with cobalt acetate and isopropyl amine. Phenacetin (acetophenetidin) and acetanilid have analgesic and antipyretic activity. Phenacetin is an ingredient in APC compound (which also contains acetylsalicylic acid and caffeine). Both give a positive indophenol test and can be distinguished by means of the isonitrile test, which is positive for acetanilid but not for phenacetin.

Several other extraction methods are available for barbiturates. However, the fast growing application of RIA has added a completely new dimension to identification of such pharmocologically active substances in plasma and tissues. These include numerous assays such as barbiturates, LSD, morphine, and amphetamines.

Hemagglutination-inhibition (HI) technique. A new technique has been successful in utilizing the hemagglutination-inhibition (HI) technique for detection of barbiturates. A barbiturate derivative is conjugated to bovine gamma globulin. This complex produces antibodies against the barbiturate moiety (portion). A new haptene synthesized is 5-ethyl-5-1-carboxy-n-propyl barbituric acid. Thus, the antibody prepared against this hapten-protein conjugate reacts with sodium barbiturate, secobarbital, phenobarbital, pentobarbital, amobarbital, and aprobarbital. Serum, as well as urine, may be utilized for the performance of the test.

Procedure. The test is performed by the following steps:

a. Dilute one drop of urine (1:10 or 1:5) with buffer or dilute serum 5 X with physiological saline.

b. Place one drop of urine (or serum) plus one drop of antiserum into a well of a titer tray.

c. After a few minutes, add the sensitized cells. The test is read in approximately 1 hour.

Alkaloids. This group can be extracted with alkaline ether and includes strychnine, amphetamines, morphine, and many others. Differentiation is quite involved and difficult. However, there is a rapid test which may be performed on urine to detect large doses of alkaloids in general. A reagent for this purpose is prepared as follows:

Mercuric iodide (red)	3 g
Potassium iodide	2 g
Glacial acetic acid	20 ml
q.s. to 60 ml with distilled H_2O	

Add 5 to 10 drops of mercuric aciodide reagent to a few milliliters of urine in a test tube. The immediate appearance of a slight turbidity indicates the presence of albumin or alkaloids. Heat the tube gently for differentiation. If the turbidity is due to alkaloids, the precipitate completely dissolves upon heating. A hemagglutination-inhibition (HI) technique for the detection of morphine in body fluids is also available commercially. This technique makes feasible the determination of the use of heroin by an addict within as many as 72 to 96 hours following the injection of heroin. This sensitivity also tends to defeat attempts to foil detection by the intake of large volumes of liquids in an attempt to dilute the morphine present in the urine.

There is also a hemagglutination procedure available for the detection of methadone. The tests for morphine and methadone are performed in similar manner as the one for barbiturates.

Heavy Metals. Each of the heavy metals is tested for separately. Most significant are arsenic, mercury, and lead. Of these, lead may have special significance to environmental health laboratories. Lead is a very common metal used in petroleum additives, storage batteries, ammunitions, etc. You are encouraged to investigate procedures for the determination of heavy metals and also the special instructions issued by reference laboratories regarding referral of specimens for heavy metal studies. Special attention is necessary to avoid contamination in the case of urinary lead studies. Atomic absorption spectroscopy is the preferred method for lead determination.

Exercises (431):

1. Which of the known drugs of abuse cannot be readily detected in body fluids?

2. What two methods are given for separation of the drug from urine?

3. What is the principle of the hemagglutination technique for detection of barbiturates?

4. What is the preferred method for lead analysis?

5. In the routine TLC process for drug determination, what is done with the thin-layer chromatographic sheets after development?

6. In a rapid test described for urinary alkaloids, what other abnormal constituent besides alkaloids gives a positive test?

7. How may these two substances be distinguished?

8. Strychnine, amphetamines, and morphine belong to which of the chemical groups of toxic compounds discussed?

432. Indicate the procedures for shipping specimens to referral laboratories by citing method of packing, container used, type and amount of specimen, directives to be followed, and forms to be used.

Tissue. Tissue specimens should be frozen solid in dry ice. Great care must be taken to avoid contaminating tissue specimens with formaldehyde, as it interferes with various toxicological studies. Specimens that may be submitted for study include bone marrow, hair, brain, muscle, and in fact practically any body tissue or body fluid. If fluid is aspirated into a container at autopsy, the container and apparatus must be free from interfering contaminants. Specimens taken should be adequate. Remember, it is always better to submit too much specimen to a consultant laboratory than too little. A list of suggested specimens and the amount to submit for analysis is presented in table 4-1. These and other toxicological specimens may be forwarded to the Epidemiology Division, USAF School of Aerospace Medicine (USAFSAM), Brooks AFB, Texas 78235. If more practicable, specimens may also be sent to the appropriate regional laboratory having the capability to perform toxicological studies. In addition to the specimen, include a pertinent medical history with the specimen from each case requiring forensic toxicology

TABLE 4-1
COLLECTION AND SUBMISSION OF TOXICOLOGICAL SPECIMENS

Poison Suspected	*Specimen*	*Minimum Quantity*	*Remarks*
Alcohols (methyl, ethyl, isopropyl)	Blood, oxalated Brain	5 ml 500 gm	Add 10 mg sodium fluoride per ml blood.
Sedatives, hypnotics (barbiturates, phenothiazines, Doriden, Meprobromate, bromides)	Blood Urine Gastric content Brain Liver	5 ml serum for each drug All available First washing 100 gm 100 gm	For barbiturates, blood is preferable to urine.
Alkaloids (narcotics)	Urine Liver Brain Kidney	200 ml 200 gm 200 gm 1 kidney	
Salicylates	Blood Urine	5 ml serum 25 ml	
Amphetamines	Urine	100 ml	
Metals (arsenic, beryllium, cadnium, chromium, copper, lead, mercury, selenium, thallium, uranium, zinc)	Urine Kidney Liver Hair (arsenic) Finger and toenails (arsenic) Blood, oxalated (lead) Serum (zinc, copper)	200-ml aliquot 24-hr collection (400 ml for uranium, beryllium) 50 gm 50 gm 0.5 gm mininum 0.5 gm minimum 5 ml 5 ml	Clean plastic containers are preferable to glass.
Fluorides	Urine Liver	100 ml 25 gm	
Cyanides	Gastric content	All available	
Carbon monoxide	Blood, oxalated/citrated	5 ml	
Inhaled poisons	Lung	1 lung	
Cholinesterase inhibitors (organic phosphorus insecticides)	Serum Blood, oxalated	2 ml 5 ml	Can be done on either serum or red cells.
Unknown poison suspected at autopsy	Gastric content Liver Kidney Blood Urine Brain Lung	All available 500 gm 1 kidney All blood clots available All available One hemisphere Both lungs	1. Do not add formalin or otherwise fix tissue. 2. Each organ in separate plastic bag; labeled.
Antihistamines (Benadryl, Pyribensamine)	Urine	50 ml	
Anticonvulsants (Dilantin, Mesantoin)	Serum	5 ml	
Chlorinated organic insecticides (DDT, Lindane)	Gastric content Body fat	First washing 10 gm	These substances are slowly absorbed from the stomach, stored in body fat.

analysis. Prepare DD Form 1323, Toxicological Examination—Request and Report, in triplicate for this purpose. This could save many days of unnecessary testing. Tissue specimens are best preserved, with few exceptions, with dry ice. If an autopsy is performed, one-half brain specimen, 500 g liver, one whole kidney, and all available blood and urine must always be forwarded. For additional instructions consult AFR 161-12, *USAF Epidemiological Services*.

Aircraft Accidents. In cases of aircraft accident facilities, all facilities must send fixed and frozen specimens for toxicological examination directly to the Armed Forces Institute of Pathology, Washington, D.C., in accordance with AFR 160-55, *The Armed Forces Institute of Pathology and Armed Forces Histopathology Centers*.

Exercises (432):

1. How should tissue specimens be shipped for toxicological analysis?

2. If you were directed to ship a urine specimen to the Epidemiology Division for arsenic determination, how much would you send? What kind of container would you use?

3. What type of specimen would you ship to the "Epi Lab" for cyanides? What amount?

4. In a case requiring forensic toxicology analysis, what form should you prepare to be sent along with the specimen?

5. What Air Force regulation provides guidelines for consultant and referral capability for laboratory diagnostic procedures that are impractical to perform at USAF medical centers?

6. What Air Force regulation provides guidance for shipment of specimens resulting from an aircraft accident?

4-3. Review of the Actions of Poisons

As you know from the previous discussion and from your own experience, a poison is a substance which may produce death, serious illness, or harmful effects when it is introduced into the body in a relatively small quantity. In concluding this chapter, let us briefly consider some basic concepts in toxicology, particularly with reference to the general effects of poisons on the body. We have already considered in some detail the specific effects of such poisons as alcohol, carbon monoxide, and salicylates.

423. Identify the effects of poisons on the body and their classification.

Effects of Poisons. The effects of poisons may be local or remote, and some poisons have both a local and a remote effect. *Local effect* means direct action on the part to which the poison is applied, such as corrosion and irritation. *Remote effect* means the action of the poison on some organ remote from the seat of application or point of introduction. Sometimes a poison shows no effect, or only a slight one, until several doses have been taken. Then an effect is produced which nearly equals that produced by taking the whole amount at one time. This is known as a *cumulative effect*.

The effect of a poison depends not solely on its concentration, but also on its solubility, the method of its introduction into the body, and the rapidity of its absorption into the system. The method of introduction may determine its toxicity. For example, snake venom taken into the mouth and perhaps even into the stomach during first-aid treatment of snakebite is not ordinarily harmful, but snake venom injected hypodermically is extremely poisonous.

There are various ways in which poisons may be introduced into the body, the most common being by mouth, inhalation, and injection. Poisons taken by mouth enter the circulation through absorption from the stomach and intestine, and those inhaled enter the circulation through the air passages and lungs. When they are introduced by hypodermic injection, poisons enter the circulation through absorption from the body tissues. If the injection is intravenous, the poisons are introduced directly into the bloodstream. Poisons may also be introduced by application to open wounds and to the unbroken skin. After entering the circulation, a poison is carried by the blood to the tissues and organs susceptible to its action.

Most of the elimination of poisons from the body takes place in the kidneys, lungs, liver, gastrointestinal tract, skin, and salivary glands. Poisons may be excreted from the system unchanged or in the form of other compounds into which they have been transformed by the action of the various body organs and tissues. The most damaging effects of some poisons are found at the points of excretion, as in the kidneys and colon in poisoning by mercuric chloride (bichloride of mercury). Various conditions of the individual may modify the actions and effects of poisons on the body. The age of the person makes a great deal of difference; young children are far more susceptible to poisons than adults. Conditions caused by poisons vary because of personal idiosyncrasy; that is, some persons are by nature unusually sensitive to certain poisons, while others possess a natural tolerance for certain poisons that is not the result of habitual use. Through habitual use of certain poisons, especially the narcotics, most persons may become so accustomed to their effects that they are not poisoned when taking doses that would ordinarily prove lethal in the unaddicted. It occasionally happens, however, that continual external use of chemical substances results in hypersensitivity.

The actions of poisons may be considerably modified by disease; some diseases increase and others lessen the action of poisons. In the latter case, large doses are usually required to produce the desired effect.

Poisoning may be either acute or chronic. *Acute poisoning* is the condition brought on by taking one overdose of a poison. *Chronic poisoning* is the condition brought on by taking repeated doses of a poison or as the result of the absorption of the poison over a long period of time. Many industrial workers, instrument assembly technicians, fuel handlers, and painters are some of the occupational groups subject to chronic poisoning. As a laboratory technician, you are also exposed to a variety of poisonous chemicals.

Classification of Poisons. Poisons may be classified according to their mode of action in a manner similar to that discussed in the previous section of this chapter. Although of limited use in the clinical laboratory, a clinical classification is helpful because it describes how poisons act. For example, poisons may be described under the headings of corrosives, irritants, neurotics, and gases.

Corrosives. Corrosives are substances which rapidly destroy or decompose the body tissues at the point of contact. When they are ingested, there is immediate burning pain in the mouth with severe burning pain in the esophagus and stomach. Swallowing is very difficult, respiration is impeded, and vomiting is inevitable. Examples are strong acids and alkalis.

Irritants. Irritant poisons are those agents which do not directly destroy the body tissues but set up an inflammatory process at the site of application or contact. Some examples are potassium nitrate, zinc chloride, zinc sulfate, ferrous sulfate, silver nitrate, arsenic, iodine, and phosphorus. General symptoms include nausea, vomiting, purging (frequently the vomited matter and stools contain blood), and pain and cramps in the abdomen. In some cases, there is inflammation of the urinary tract.

Neurotics. Neurotics are poisons which act on the brain, spinal cord, and the central nervous system. Some examples are opium, hydrocyanic acid (prussic acid), ether, chloroform, aconite, strychnine, belladonna, ethyl and methyl alcohol, LSD, and the barbiturates. Symptoms may be divided into two subclasses:

a. Depressants, which produce symptoms characterized by a period of exhilaration followed by drowsiness and stupor; slow and stertorous breathing; cold, clammy skin; cyanosis; slow pulse; muscular relaxation; dilated or contracted pupils; and insensibility to external impressions.

b. Excitants, which produce symptoms characterized by rapid and feeble pulse, delirium, hot and dry skin, a sense of suffocation and the inability to breathe, shuddering and jerking of muscles, dilated or contracted pupils, disordered vision, and sometimes convulsions and tetany (as in the case of strychnine poisoning).

Gaseous poisons. These are poisons present in the gaseous state which, if inhaled, destroy the oxygen-carrying property of the blood. They irritate the tissues of lungs and air passages, and, if in contact with the skin or mucous membranes, are highly irritating. Symptoms include irritation and corrosion of the respiratory tract, with resultant bronchitis, and irritation of the eyes, mouth, stomach, and kidneys.

Food poisoning. This chapter would be incomplete without at least mentioning food poisoning. You will, of course, study this topic in greater detail in a subsequent volume relating to microbiology. Food poisoning can cause acute attacks of illness in more persons in a short time than any other agent. The term "food poisoning" is conventionally divided into two types: food intoxication and food infection.

a. Food intoxication is due to a specific toxin produced outside the body; for example, the toxin of botulism. Other organisms also cause food intoxication by producing toxins, the exact nature of which is imperfectly understood. These toxins are formed under suitable conditions usually by Staphylococcus, occasionally by Streptococcus, and rarely by coliform and Proteus species.

b. Food infection usually is caused by a specific group of organisms, namely the Salmonella group, and occasionally by the dysentery group. Gastrointestinal distress, nausea, vomiting, diarrhea, urticaria, and circulatory and nervous disturbances are the general symptoms of food poisoning, and they may vary from mild discomfort to violent disturbances of normal functions of the body. In more severe forms the neurologic symptoms may overshadow the gastrointestinal symptoms, followed by relapse. Death is usually due to respiratory paralysis, cardiac failure, or secondary pneumonia.

Exercises (433):

Match the following by placing the letter of the column B item beside the column A item or items that most nearly describe it. Each element in column B may be used once.

Column A

___ 1. Food poisoning due to specific toxin produced outside the body, such as that produced by microorganisms.

___ 2. Sometimes a poison shows no effect or only a slight one until several doses have been taken.

___ 3. Refers to the presence of toxic-producing agents in the body and the consequent effects of their presence.

___ 4. The action of the poison on some organ that is distant from the seat of application or point of introduction.

___ 5. Direct action on the part to which the poison is applied, such as corrosives and irritants.

___ 6. Condition brought about by taking one overdose of a poison.

___ 7. Many industrial instrument assembly technicians, fuel handlers, painters, and even laboratory technicians are most susceptible to this type of poisoning.

___ 8. Substances which rapidly destroy or decompose the body tissues at point of contact.

___ 9. Poisons which act on the brain, spinal cord, and central nervous system, such as LSD, methyl and ethyl alcohol, and the barbiturates.

___ 10. These produce symptoms characterized by a period of exhilaration followed by drowsiness and stupor, and slow and stertorous breathing.

___ 11. They irritate the tissues of the lungs and air passages and mucous membranes. Symptoms include irritation and corrosion of respiratory tract resulting in bronchitis.

___ 12. These do not directly destroy the body tissues, but set up an inflammatory process at the site of application or contact.

Column B

a. Local effect.
b. Remote effect.
c. Cumulative effect.
d. Acute poisoning.
e. Chronic poisoning.
f. Corrosives.
g. Irritants.
h. Neurotics.
i. Depressants.
j. Gaseous poisons.
k. Food intoxication.
l. Food infection.

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NOTE: None of the items listed in the bibliography above are available through ECI. If you cannot borrow them from local sources, such as your base library or local library, you may request one item at a time on a loan basis from the AU Library, Maxwell AFB, Alabama, ATTN: ECI Bibliographic Assistant. However, the AU Library generally lends only *books* and a limited number of *AFM's.* TO's, classified publications, and other types of publications are *not* available.

Answers for Exercises

CHAPTER 1

Reference:

400 - 1. (1) Maintenance of acid-base and electrolyte balance, (2) excretion of waste products of metabolism, (3) maintenance of osmotic equilibrium, and (4) excretion of foreign substances such as dyes.

400 - 2. (1) The rate of renal blood flow, (2) the activity of the kidney tubules, and (3) the rate and efficiency of glomerular filtration.

400 - 3. (1) Clearance tests, (2) excretion tests, (3) concentration-dilution tests, and (4) retention tests.

401 - 1. (1) Direct condensation of urea with diacetyl to form a measurable chromogen, (2) indirect determination of ammonia as a product of urease action on urea, and (3) miscellaneous photometric and physical procedures.

401 - 2. It does not determine NH_3 (ammonia) and reacts directly with urea as well as with dibasic amino acids and peptides in the blood.

401 - 3. (1) Rapid development and fading of color, (2) photosensitive color, (3) color that does not follow Beer's law with filter photometer and spectrophotometer, (4) unpleasant and irritant fumes requiring working in a fume hood, (5) heating time for maximal color development that is dependent on the urea concentration, and (6) the reaction is not completely specific.

401 - 4. The enzyme urease is used to hydrolyze urea to ammonium carbonate, which is nesslerized and measured photometrically with a suitable standard.

401 - 5. Urea in the specimen is converted to ammonia by the enzyme urease in the buffered solution. Upon addition of sodium phenate and hypochlorite, a stable blue color is developed proportional to the concentration of ammonia.

401 - 6. Increase results.

401 - 7. They interfere with enzyme activity and will lower the results.

401 - 8. It does not significantly affect the results.

402 - 1. Occuring as the end product of purine metabolism, uric acid is a component of the total nonprotein nitrogen of the body.

402 - 2. Elevated levels are due to renal disorders, blood dyscrasias, lead poisoning, liver disease, or gout.

402 - 3. Hexavalent phosphotungstic acid is reduced by uric acid to a lower valence in alkaline solution with the formation of a blue color.

402 - 4. As a source of alkali.

402 - 5. Males: 2.0-8.0 mg per dl.
Females: 1.0-7.0 mg per dl.

402 - 6. No particular significance.

402 - 7. Creatinine is one of the nonprotein nitrogens containing constituents of blood and has the formula $C_4H_7ON_3$.

402 - 8. Creatinine in the PFF reacts with picric acid in an alkaline solution to form creatinine picrate, which is a yellow to red color.

402 - 9. Creatine is the anhydride of creatinine. Unlike creatinine, creatine is not a waste product of metabolism, but is derived from glycine, arginine, and methionine.

402 - 10. First the creatinine is assayed and then the creatine is converted to creatinine. Creatinine is assayed again, and the difference after correction for differences in molecular weight represents the creatine level.

403 - 1. 15 minutes, 30 minutes, 60 minutes, and 120 minutes.

403 - 2. BSP dye, if PSP is done within 24 hours of injection; bile; hemoglobin.

403 - 3. Because PSP dye is supplied in vials of 1 ml each, this exact amount is injected (some vials may contain slightly more to allow for loss in drawing the dye into a syringe).

404 - 1. After it is filtered by the glomeruli, it is partially reabsorbed by the tubules.

404 - 2. The rate of urine flow, the amount of urea present, and the state of the tubular epithelium.

404 - 3. Increased level.

404 - 4. An index to glomerular filtration rate.

404 - 5. Creatinine is not reabsorbed by the tubules; fluid intake and excretion have lesser effect on creatinine clearance.

405 - 1. Release of anti-diuretic hormone (ADH) by pituitary in water deprivation, reabsorption of sodium caused by aldosterone, with the amount of urea excreted by the kidney contributing to changes in levels of specific gravity and osmolality.

405 - 2. Specific gravity; osmolality.

405 - 3. Determines the freezing point depression concentration of free particles in a solution; milliosmol.

405 - 4. The patient ingests more fluid than allowed.

405 - 5. 800-1300 mOsm/liter; up to 300 ml; 1.022-1.032.

405 - 6. Dilution test.

405 - 7. At least one specimen should fall below 80 mOsm/liter and may decrease to 37 mOsm/liter.

CHAPTER 2

406 - 1. c.

406 - 2. a.

406 - 3. b.

406 - 4. e.

406 - 5. h.
406 - 6. j.
406 - 7. f.
406 - 8. d.
406 - 9. g.
406 - 10. i.

407 - 1. HCl is formed in the parietal cells of tubular glands from plasma chloride ions and hydrogen ions from the dissociation of H_2CO_3. The cells secrete the acid through gastric pits in the mucosa.
407 - 2. 0.9 to 1.5.
407 - 3. e.
407 - 4. a.
407 - 5. b.
407 - 6. d.
407 - 7. c.
407 - 8. f.

408 - 1. The concept of "free" and "combined" as a part of the total acid has been shown to have neither physiologic nor physicochemical validity.
408 - 2. (1) Volume, (2) titratable acidity, and (3) pH.
408 - 3. It is expressed in milliequivalents per liter. Each sample value is calculated by multiplying its volume by the titratable acidity and dividing by 1000.
408 - 4. A 1-hour collection is made consisting of four 15-minute samples. The basal acid output is determined and reported in milliequivalents as the sum of the acid outputs for the four samples.
408 - 5. The value of the total gastric secretion collected by continuous aspiration in four 15-minute samples for 1 hour after injection of histamine or Histalog test. Value is expressed in milliequivalents.
408 - 6. As defined by most authorities, anacidity is a failure of the pH to fall below either 6.0 or 7.0 in the augmented histamine or Histalog tests.
408 - 7. A condition in which gastric-secreting pancreatic tumors bombard the parietal cells with continuous high-level stimulation of high acid output.
408 - 8. The terms have such fine lines of distinction that they have little clinical significance.

409 - 1. T.
409 - 2. F. It is helpful.
409 - 3. F. A slight gray to pale yellow color.
409 - 4. T.
409 - 5. F. Blood accumulated in the stomach.
409 - 6. T.

410 - 1. 0.1N NaOH.
410 - 2. Measure the volume of the specimen and determine the pH with a suitable pH meter.
410 - 3. pH 1.68 and pH 7.0.
410 - 4. Using phenol red indicator, 0.1 percent aqueous solution.
410 - 5. Add water and calculate the dilution factor in computing titratable acidity.
410 - 6. It is expressed in milliequivalents per hour, and may be calculated by dividing the total amount secreted by the collection time in hours (mEq/hr).
410 - 7. A dye, such as azure-A, attached to an ion exchange resin is given to the patient orally. The amount of dye that appears in the urine is measured as an index of the amount of HCl that released dye in the stomach.
410 - 8. This is generally accepted as a valid screening procedure for determining whether hypoacidity or hyperacidity exists.

CHAPTER 3

411 - 1. d.
411 - 2. f.
411 - 3. h.
411 - 4. i.
411 - 5. b.
411 - 6. c.
411 - 7. j.
411 - 8. g.
411 - 9. e.
411 - 10. a.

412 - 1. T.
412 - 2. F. Blot it between filter papers to remove excess moisture, but keep it moist.
412 - 3. T.
412 - 4. T.
412 - 5. F. Reading from anode to cathode on the strip are albumin, alpha 1, alpha 2, beta, and gamma (α_1, α_2, β, and γ)
412 - 6. F. Specimens are stable for 3 days at room temperature and 1 month at refrigerated temperature.
412 - 7. T.
412 - 8. F. Since they have identical migration speeds, they cannot be differentiated.
412 - 9. T.
412 - 10. T.
412 - 11. F. Starch block electrophoresis.
412 - 12. F. The medium of choice is cellulose acetate.
412 - 13. T.

413 - 1. b.
413 - 2. c.
413 - 3. d.
413 - 4. a.
413 - 5. e.
413 - 6. f.

414 - 1. a.
414 - 2. a.
414 - 3. c.
414 - 4. b.
414 - 5. c.
414 - 6. b.
414 - 7. b.
414 - 8. d.
414 - 9. d.

415 - 1. Serum iron is the clinically significant iron, other than that normally present as hemoglobin, which is loosely bound to siderophilin for transport in the plasma.
415 - 2. Hypochromic anemia and various infectious diseases.
415 - 3. Normal values range from 65 to 175 μg percent.
415 - 4. Unsaturated iron-binding capacity (UIBC) or latent iron-binding capacity (LIBC).
415 - 5. Serum iron plus the unsaturated iron-binding capacity (UIBC) are the components of the total iron-binding capacity.
415 - 6. It simultaneously releases the iron and precipitates protein.

416 - 1. F. Inorganic iodine makes up about 10 percent of the iodine in serum.
416 - 2. T.
416 - 3. F. Thyroxine is the protein-bound iodine (PBI).
416 - 4. T.
416 - 5. F. The simpler method uses an ion-exchange resin that will absorb the organic iodide but not the PBI.
416 - 6. T.
416 - 7. T.
416 - 8. F. The rate of reaction or change in optical density rather than a stable optical density determines the concentration thus obtained.
416 - 9. F. Normal values are generally 4 to 8 μg/100 ml.
416 - 10. T.

337

416 - 11. F. This is not a problem with the alkaline ash procedure.
416 - 12. F. Sulfuric acid, nitric acid, and perchloric acid are employed in the Technicon procedure.

417 - 1. Column chromatography.
417 - 2. Ammonia.
417 - 3. A stronger solution of acetic acid (pH 1.4).
417 - 4. First eluate—T_4 (iodine)—at least 70 percent. Second eluate T_4—30 percent.
417 - 5. Acetic acid solution containing T_4 treated with bromine water can be used directly in the ceric-arsenite reaction without the necessity of wet ashing.
417 - 6. 3.2 to 7.2 μg/dl.
417 - 7. 5.0 to 11.0 μg/dl.
417 - 8. Endogenous and exogenous.

418 - 1. h.
418 - 2. f.
418 - 3. g.
418 - 4. d.
418 - 5. e.
418 - 6. c.
418 - 7. b.
418 - 8. a.

419 - 1. T.
419 - 2. F. Estrogens are formed in the ovaries, the adrenal cortex, and the placenta.
419 - 3. F. Bioassy, colorimetric, and fluorometric methods are either nonspecific or too involved to be performed in a clinical laboratory.
419 - 4. T.
419 - 5. T.
419 - 6. F. Decreased estrogen levels are evidence of a complicated pregnancy; increased values are most commonly associated with tumors of the ovaries.
419 - 7. F. A 24-hour urine sample is required.
419 - 8. T.
419 - 9. F. Pregnandiol is the reduction product and the form in which progesterone is usually analyzed.
419 - 10. F. Pregnandiol is determined by column chromatography.
419 - 11. F. Lower pregnandiol values are associated with threatened abortion or toxemia of pregnancy.
419 - 12. T.
419 - 13. F. The best technique is radioimmunoassay.
419 - 14. T.

420 - 1. a.
420 - 2. g.
420 - 3. f, g.
420 - 4. e.
420 - 5. h.
420 - 6. e.
420 - 7. c.
420 - 8. g.
420 - 9. c.
420 - 10. c.
420 - 11. f.
420 - 12. c.
420 - 13. c.
420 - 14. f.
420 - 15. d.

421 - 1. The gonadotropic hormone.
421 - 2. It stimulates the ovarian follicle to increase in size and to mature. In the male, it is significantly involved in the stimulation and maintenance of spermatogenesis.
421 - 3. It causes ovulation and steroid production such as estrogen and progesterone by the corpus luteum. It also stimulates the interstitial cells to produce androgen and estrogen.
421 - 4. 24-hour urine specimen.
421 - 5. Serum or plasma.
421 - 6. A mouse unit is defined as the least amount of estrus-producing gonadotropin which induces desquamation of the vaginal epithelium in the spayed mouse.

422 - 1. a.
422 - 2. c.
422 - 3. a.
422 - 4. d.
422 - 5. e.
422 - 6. u.
422 - 7. t.
422 - 8. v.
422 - 9. r.
422 - 10. s.
422 - 11. p.
422 - 12. w.
422 - 13. k.
422 - 14. n.
422 - 15. l.
422 - 16. j.
422 - 17. i.
422 - 18. h.

423 - 1. Carcinoid tumors.
423 - 2. First, tryptophan is oxidized and then decarboxylated to form serotonin.
423 - 3. Argentaffin cells of the intestinal mucosa.
423 - 4. Large amounts are found in the urine in such cases.
423 - 5. Nitrous acid and 1-nitroso-2-naphthol with 5-HIAA.
423 - 6. 2, 4 dinitrophenylhydrazine for removal of interfering keto acid and chloroform extraction to remove the indoloacetic acid.
423 - 7. Eggplant, avocados, bananas, pineapple, and plums.
423 - 8. 10 ml HCl as a preservative and for maintaining an acid pH during collection.
423 - 9. F. Epinephrine and norepinephrine.
423 - 10. T.
423 - 11. T.
423 - 12. T.
423 - 13. T.
423 - 14. F. To remove most urinary chromogens.
423 - 15. T.
423 - 16. F. They require a spectrophotometer in the near ultraviolet range.
423 - 17. F. The normal values are 0.7 to 6.8 mg/24 hours.
423 - 18. F. The antibiotic nalidixic acid (NegGram) increases the VMA value.

424 - 1. Gallbladder, prostate, tonsils, and salivary glands.
424 - 2. (1) Aid the physician in preventing future stone formation, and (2) aid in diagnosing the underlying disease.
424 - 3. Calcium oxalate stones and mixed stones of calcium oxalate and carbonate or phosphate salt.
424 - 4. F. The calculi are examined by reflected light or by polarized light.
424 - 5. T.
424 - 6. T.
424 - 7. F. Chemical analysis is the most widely used technique.
424 - 8. T.
424 - 9. F. The optical technique is affected by opaque X-ray impurities.
424 - 10. T.

CHAPTER 4

425 - 1. A clinical evaluation of an individual's state of intoxication constitutes a sobriety test. A blood alcohol

determination is merely a laboratory procedure to determine the level of alcohol in the blood.
425 - 2. UCMJ, Article 31.
425 - 3. No. Tinctures contain alcohol, and this is a possible source of contamination.
425 - 4. A chain of custody receipt is a log showing by whom and how a specimen is handled from the time it is drawn until the test is run. It is important as evidence that a specimen was properly safeguarded, a factor to be considered if the report is used in a court of law.
425 - 5. It should be sent by registered mail.
425 - 6. Reports may be given only to a concerned medical officer or to someone designated by the commander.
425 - 7. Reports of a blood alcohol test are *never* released out of medical channels by laboratory personnel without specific authorization to do so.

426 - 1. F. Ethyl alcohol is also known as grain alcohol.
426 - 2. F. A 50-proof whiskey is a 25-percent solution.
426 - 3. T.
426 - 4. F. It can be detected in tissue and all other body fluids, including CSF.
426 - 5. T.
426 - 6. F. C_2H_5OH is transformed into acetaldehyde.
426 - 7. T.
426 - 8. T.
426 - 9. F. Other organic substances formed are destructive to kidney and liver tissue.
426 - 10. F. A person is considered "under the influence" at a blood concentration of 150 mg-% (1.5 mg/ml).
426 - 11. T.
426 - 12. F. The overall effect is that of a depressant.
426 - 13. T.
426 - 14. T.
426 - 15. F. Methyl alcohol is far more toxic than ethanol.
426 - 16. F. There is little physical difference between ethanol and methanol; this frequently results in mistaken use.

427 - 1. c.
427 - 2. c.
427 - 3. f.
427 - 4. a.
427 - 5. g, i.
427 - 6. b.
427 - 7. b.
427 - 8. b.

428 - 1. An antipyretic reduces temperature, and an analgesic relieves pain.
428 - 2. Salicylates act on the hypothalmus, an area of the brain which functions as a biological thermostat.
428 - 3. Aspirin is acetyl salicylic acid, a salicylate ester of acetic acid.
428 - 4. Salicylates cause respiratory alkalosis from increased respiration following respiratory acidosis from increased CO_2 production. Metabolic acidosis results from impaired renal function and the loss of bicarbonate.
428 - 5. (1) Oxidative phosphorylation, (2) carbohydrate metabolism, (3) nitrogen balance, (4) fat metabolism, and (5) enzymes.
428 - 6. Salicylic acid reacts with acidified ferric nitrate to form a purple iron complex.
428 - 7. Precipitates the protein.
428 - 8. Acetoacetic acid and diacetic acid.
428 - 9. By boiling.
428 - 10. 50 mg per dl.

429 - 1. Carbon monoxide is a colorless, odorless, flammable gas, slightly lighter than air.
429 - 2. Carbon monoxide has a greater affinity for hemoglobin than oxygen has. Consequently, the formation of carboxyhemoglobin results in anoxia or unavailability of oxygen to body tissues.
429 - 3. (1) Concentration of carbon monoxide in the atmosphere, (2) time of exposure, (3) age and physical condition of the patient, especially his hemoglobin level.
429 - 4. 0 to 5 percent.
429 - 5. Carbon monoxide, acid-liberated from a blood sample, reacts with palladium chloride in a microdiffusion cell, causing the release of metallic palladium which forms a "mirror" on the surface of the reagent.
429 - 6. Oxyhemoglobin is reduced by sodium hydrosulfite.
429 - 7. The result should still be reliable if the test is properly performed.

430 - 1. d.
430 - 2. c, f, g.
430 - 3. e.
430 - 4. h.
430 - 5. b.
430 - 6. a.

431 - 1. Hallucinogens (LSD), marijuana, and mescaline.
431 - 2. Extraction method and nonionic exchange resin.
431 - 3. A barbiturate derivative conjugated to bovine gamma globulin which produces antibodies against the barbiturate portion. The antibody prepared will react against sodium barbiturate, secobarbital, phenobarbital, and aprobarbital.
431 - 4. Atomic absorption spectroscopy.
431 - 5. Sheets are examined under ultraviolet light and sprayed with a number of reagents in sequence to develop colors that differ for different types of drugs. Drugs are identified by their color values in comparison to known substances on the same sheet.
431 - 6. Albumin.
431 - 7. When it is heated, the precipitate dissolves completely if it is due to alkaloids.
431 - 8. Alkaloids.

432 - 1. Frozen solid in dry ice.
432 - 2. 200 ml; clear plastic container.
432 - 3. Gastric; all available.
432 - 4. DD Form 1323, Toxicological Examination—Request and Report.
432 - 5. AFR 161-12, *USAF Epidemiological Services.*
432 - 6. AFR 160-55, *The Armed Forces Institute of Pathology and Armed Forces Histopathology Centers.*

433 - 1. k.
433 - 2. c.
433 - 3. l.
433 - 4. b.
433 - 5. a.
433 - 6. d.
433 - 7. e.
433 - 8. f.
433 - 9. h.
433 - 10. i.
433 - 11. j.
433 - 12. g.

STOP-

1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.

2. USE NUMBER 2 PENCIL ONLY.

90411 03 23

EXTENSION COURSE INSTITUTE
VOLUME REVIEW EXERCISE
LABORATORY PROCEDURES IN CLINICAL CHEMISTRY (PART II)

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that item numbers on answer sheet are sequential in each column.
3. Use a medium sharp #2 black lead pencil for marking answer sheet.
4. Write the correct answer in the margin at the left of the item. (When you review for the course examination, you can cover your answers with a strip of paper and then check your review answers against your original choices.) After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if a all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DONT'S:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than a #2 black lead pencil.

NOTE: NUMBERED LEARNING OBJECTIVE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Learning Objective Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Learning Objectives* indicated by these *Numbers.* The VRE results will be sent to you on a postcard which will list the *actual* VRE *items you missed.* Go to the VRE booklet and locate the *Learning Objective Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (400) Which of the following is not an essential function of the kidney?

   a. Maintenance of osmotic equilibrium.
   b. Maintenance of acid-base and electrolyte balance.
   c. Excretion of foreign substances such as dyes, poisons, or drugs.
   d. Detoxification of foreign substances, such as dyes, poisons, and drugs.

2. (400) Activity of the kidney tubules can be measured by use of

   a. concentration and dilution tests.
   b. dye excretion tests.
   c. clearance tests.
   d. retention tests.

3. (401) What would be the value of the urea if the BUN value was 20 mg/dl?

   a. 4.280 mg/dl.
   b. 43.80 mg/dl.
   c. 42.80 mg/dl.
   d. 41.80 mg/dl.

4. (401) Which of the following is not usually classified as a method used for the determination of urea?

   a. Indirect determination of ammonia as a product of urease action on urea.
   b. Direct determination of ammonia as a product of urease action on urea.
   c. Direct condensation of urea with diacetyl to form a measurable chromogen.
   d. Miscellaneous procedures involving various photometric or physical principles of analysis.

5. (401) One important advantage of the diacetyl monoxime method is that

   a. it does not determine ammonia and it reacts directly with ureas as well as dibasic amino acids.
   b. it determines all the NH_3 (ammonia) as well as dibasic amino acids.
   c. the color develops rapidly and it is quite stable.
   d. the reaction is completely specific.

6. (401) The normal range for a blood urea nitrogen in milligrams per dl of serum or plasma is

   a. 6 - 15.
   b. 8 - 20.
   c. 15 - 30.
   d. 20 - 30.

7. (402) Which of the following is found in uric acid?

   a. Sulfur.
   b. Protein.
   c. Ammonia.
   d. Nitrogen.

8. (402) In the method for uric acid determination by Henry et al, hexavalent phosphotungstic acid is reduced to a lower valence with formation of a blue color in

   a. alkaline solution.
   b. neutral solution.
   c. acid solution.
   d. ammonia.

9. (402) The function of creatine in the body is best described as

   a. a waste product of creatinine metabolism.
   b. functions in muscle contraction in the form of phosphokinase.
   c. functions in muscle contraction in the form of phosphocreatine.
   d. a waste product of creatinine derived from glycine, arginine, and methionine.

10. (403) Which of the following substances will not invalidate the PSP test?

a. Bile.
b. Urates.
c. Hemoglobin.
d. BSP dye for first 24 hours.

11. (404) The creatinine clearance test is more accurate than is the text for urea clearance because

a. it is influenced by so many variables.
b. the amount of creatinine present is dependent on the protein content of the diet,
c. creatinine is not reabsorbed by the tubules and is affected least by fluid intake and excretion.
d. creatinine is reabsorbed by the tubules and is affected more by the fluid intake and excretion.

12. (405) Concentration tests are dependent on all of the following factors except

a. the amount of urea excreted by the kidneys.
b. reabsorption of sodium caused by aldosterone.
c. the total urinary output caused by aldosterone.
d. release of antidiuretic hormone (ADH) by the pituitary in water deprivation.

13. (405) The osmometer is an instrument that

a. determines the concentration of free particles in a solution by measuring the freezing point.
b. determine the concentration of bound particles in a solution by measuring the freezing point.
c. determines the indirect estimation of the refractive index.
d. is calibrated in terms of total solids.

14. (405) A patient with adrenal insufficiently would probably have a urine output which is

a. normal.
b. slightly decreased.
c. significantly increased.
d. significantly decreased.

15. (406) The cells of the stomach which secrete HCl are

a. rugae.
b. chief cells.
c. mucous cells.
d. parietal cells.

16. (407) The surgical removal of the stomach is known as a

a. cholecystectomy.
b. gastrectomy.
c. gastrotomy.
d. gastricism.

17. (407) A mucoprotein, present in gastric juice, which is essential for the prevention of anemia is called

a. intrinsic factor.
b. transferrin.
c. peptidase.
d. mucin.

18. (407) HCl in the stomach is formed in the

a. gastric pits of the mucosa.
b. chief cells from plasma sodium ions and hydrogen ions.
c. columnar epithelium from plasma chloride ions and hydrogen ions.
d. parietal cells of the tubular glands from plasma chloride ions and hydrogen ions.

19. (408) Which of the following terms is not currently recommended for use in the measurement of gastric secretion?

a. pH.
b. Volume.
c. Combined acidity.
d. Titratable acidity.

20. (408) Most authorities define anacidity as

a. the absence of free acid.
b. failure of the gastric secretory pH to fall below 3.5.
c. the least acid output in two successive 15-minute periods in the augmented histamine test.
d. a failure of the pH to fall below either 6.0 or 7.0 in the augmented Histalog or histamine tests.

21. (408) Anacidity nearly always accompanies

a. aplastic anemia.
b. pernicious anemia.
c. hypochromic anemia.
d. rheumatoid arthritis.

22. (409) In what way may excessive mucus in a gastric specimen affect analysis?

a. Indicate a problem of stasis.
b. Cause falsely elevated acid values.
c. Cause falsely decreased acid values.
d. Provide ease in pipetting the sample.

23. (410) Which of the following formulas would you use to calculate the acid output?

a. $\frac{\text{Titratable acidity (mEq/L) X specimen volume}}{1000}$.

b. $\frac{\text{Titratable acidity (mg/dl) X specimen volume}}{1000}$.

c. $\frac{\text{Titratable acidity (mEq) X specimen volume}}{1000}$.

d. $\frac{\text{Titratable acidity (mg) X specimen volume}}{1000}$.

24. (410) If the specimen of gastric secretion is insufficient, you may

a. indicate "QNS" on the lab slip.
b. add water and calculate the dilution factor in computing titratable acidity.
c. add saline and calculate the dilution factor in computing titratable acidity.
d. titrate the insufficient specimen using no dilution factor in computing titratable acidity.

25. (411) The migration of charged particles suspended in an electrolyte solution under the influence of an electric current flow defines

a. nephelometry.
b. iontophoresis.
c. chromatography.
d. electrophoresis.

26. (411) Which of the following is not an advantage that cellulose acetate strip has over paper as a supporting matrix in electrophoresis?

a. Quality has great variability.
b. Can be used without special preparation.
c. Stronger than paper and easy to handle.
d. Good transparency facilitates quantitation by densitometry.

27. (412) In the fractionation order from the negative to the positive electrodes, albumin, α_1, α_2, β, and γ globulins are electrophoretic fractions of

a. hemoglobin.
b. amino acids.
c. serum protein.
d. serum lipoprotein.

28. (413) The physical property of absorption is used to separate mixtures in

a. electrolysis.
b. chromatography.
c. plasmaphoresis.
d. electrophoresis.

29. (414) What technique of chromatography is indicated when a strip is sprayed or dipped into a reagent that will give a color reaction with the substances to be determined?

a. Ion-exchange chromatography.
b. Adsorption chromatography.
c. Partition chromatography.
d. Paper chromatography.

30. (415) In the determination of serum iron, the first splitting off of the iron from its carrier protein is accomplished with

a. nitric acid.
b. acetic acid.
c. tungstic acid.
d. hydrochloric acid.

31. (416) In the colorimetric determination of PBI, the reaction involves reduction of yellow colored ceric ions to colorless cerious ions by what reagent?

a. Chloric acid.
b. Arsenious acid.
c. Hydrochloric acid.
d. Trichloroacetic acid.

32. (417) The elimination of many troublesome iodine-containing organic compounds in the first wash and the retention of inorganic iodide by the column offer greater specificity over the PBI in the determination of the

a. thyroxine by column (T_4).
b. automated analysis of the PBI.
c. butanol-extractable iodine (BEI).
d. the alkaline incineration manual method for PBI determination.

33. (418) Which of the following studies is useful with patients who have recently ingested iodides or had radiocontrast studies in which PBI values would be inaccurate?

a. Blood volume.
b. Triolein uptake.
c. T_4 or Murphy-Pattee.
d. Radioactive T_3 red cell uptake.

34. (419) Pregnandiol and pregnantriol are reduction products of

a. estrogen.
b. androgen.
c. progesterone.
d. testosterone.

35. (419) Androgens are assayed clinically by

a. the 17-ketosteroid procedure.
b. the androsterone.
c. precipitating digitonin.
d. measuring testosterone.

36. (419) Which of the following methods is considered to be the method of choice for measurement of plasma or serum testosterone levels?

a. Radioimmunoassay.
b. Gas-liquid chromatography.
c. Flame ionization detection.
d. Double-isotope derivative techniques.

37. (420) For the assay of 17-ketosteroids, some procedures may require a

a. random specimen.
b. 12-hour specimen with or without preservatives.
c. 24-hour specimen with or without preservatives.
d. 250-ml aliquot of a 24-hour specimen with 10 ml of HCl added.

38. (420) In the Porter-Silber reaction, certain compounds react with phenylhydrazine to form a yellow compound in which of the following procedures?

a. Catecholamine.
b. 17-ketosteroid.
c. 17-ketogenic steroid.
d. 17-hydroxycorticosteroid.

39. (421) All of the following statements are true concerning the FSH except one. Which statement is the exception?

a. Causes ovulation and production of steroids such as estrogen and progesterone.
b. Stimulates the ovarian follicles to increase in size and to mature.
c. Stimulates and maintains spermatogenesis.
d. Requires a 24-hour urine specimen.

40. (422) Which of the following is not a common procedural step used to make a radioimmunoassay?

a. Incubation of constant quantities of labeled antigens and antibodies with antigen.
b. Separation of bound antigen from antigen.
c. Determination of bound radioactivity.
d. Determination of bound antibodies.

41. (422) Which of the following radioimmunoassay procedures is used to diagnose and evaluate renal hypertension?

a. Renin angiotensin I.
b. Serum cortisol.
c. Thyroxine, T_4.
d. Vitamin B-12.

42. (422) Which of the following RIA procedures serves as a dianostic aid for patients with signs and clinical history consistent with carcinoma?

a. Digoxin level.
b. Serum cortisol.
c. Chorionic somatomammotropin.
d. Carcinoembryonic antigen (CEA).

43. (423) Excessive amounts of serotonin are produced from

a. carcinoid tumors of the argentaffin cells.
b. metabolic processes of the argentaffin cells.
c. metabolic processes of the gastrointestinal mucosa.
d. the breakdown by oxidative deamination by the enzyme amino oxidase.

44. (423) Photometric measurement of 5-HIAA is based on the color complex formed by 5-HIAA with

a. nitric acid and 1-nitroso-2-naphthol.
b. nitric acid and 1-nitroso-3-naphthol.
c. nitrous acid and 1-nitrose-2-naphthol.
d. 2, 4 dinitrophenylhydrazine and chloroform.

45. (423) Which of the procedures for catecholamines determination involves a technique of paper chromatography whereby chromatograms must be examined under ultraviolet light?

a. Chemical-colorimetric method.
b. Physico-chemical method.
c. Pharmacologic method.
d. Biologic method.

46. (423) One method for VMA determination involves color development with p-nitroaniline after treatment of the specimen with magnesium silicate and extraction with ethyl acetate. The solution in which the color is developed is made

a. acid with potassium carbonate.
b. alkaline with potassium carbonate.
c. alkaline with ammonium phosphate.
d. alkaline with ammonium carbonate.

47. (423) Which of the following antibiotics increases the VMA value by producing an interfering drug metabolite?

a. Neomycin.
b. Nafcillin.
c. Novobiocin.
d. Nalidixic acid.

48. (424) The technique most widely used for analysis of calculi is

a. chemical.
b. pulverization.
c. X-ray diffraction.
d. infrared spectroscopy.

49. (424) Which of the following could be found in the chloroform portion of kidney stone?

a. Indigo.
b. Uric acid.
c. Cholesterol.
d. Calcium oxalate.

50. (424) In which technique for stone analysis is part of the pulverized material subjected to a screening flame test which identifies the burning characteristics of certain groups of compounds?

a. Infrared absorption technique.
b. X-ray defraction technique.
c. Chemical analysis.
d. Optical method.

51. (425) A clinical evaluation of an individual's state of intoxication constitutes a

a. sobriety exam.
b. blood alcohol exam.
c. drug screening exam.
d. medical blood alcohol exam.

52. (425) The best guideline in resolving medical-legal difficulties associated with toxicology testing is to

a. follow the physician's written request to the letter.
b. perform only those tests which the patient approves.
c. perform your duties conscientiously and not be concerned with legal responsibilities.
d. follow local policy and when in doubt consult your supervisor and/or the Staff Judge Advocate.

53. (425) Which of the following may be used to prepare the arm prior to venipuncture for a blood alcohol test by a reduction procedure?

a. Acetone.
b. Aqueous iodine.
c. Tincture of iodine.
d. Tincture of merthiolate.

54. (425) What sort of record should you keep to show that a blood alcohol specimen was properly guarded from the time it was drawn until the test run and by whom, when, and how the specimen was handled?

a. Chemistry logbook.
b. Custodial logbook.
c. Chain of custody receipt.
d. Clinical laboratory timesheet.

55. (426) Ethyl alcohol is the same as

a. CH_3COCH_3.
b. ethanol.
c. aminoethane.
d. antabuse.

56. (426) Most of the alcohol in the blood is

a. reabsorbed in the kidney tubules.
b. rapidly excreted in the urine.
c. excreted in perspiration.
d. exhaled from the lungs.

57. (427) Aliphatic alcohols do not produce false positives to adversely influence the test results in which of the following methods for blood alcohol?

a. Dichromate.
b. Microdiffusion.
c. Gas chromatography.
d. Alcohol dehydrogenase-enzymatic.

58. (427) If postmorten blood alcohol is not carried out as soon as possible, falsely elevated results may be obtained due to

a. stasis.
b. coagulation.
c. putrefaction.
d. formaldehyde.

59. (427) Other volatile substances besides ethanol may be identified and quantitated by

a. dichromate.
b. Anstie's reagent.
c. enzymatic methods.
d. gas chromatography.

60. (428) An antipyretic commonly used therapeutically is

a. methyl salicylate.
b. acetyl salicylate.
c. lithium oxalate.
d. disulfiram.

61. (428) Trinder reagent used in the blood salicylate test contains

a. mercuric chloride, which precipitates the protein, and ferric nitrate to form the color.
b. mercuric nitrate, which precipitates the protein, and ferric chloride to form the color.
c. mercuric nitrite, which precipitates the protein, and ferric nitrate to form the color.
d. mercuric nitrate, which precipitates protein, and ferrous nitrate to form the color.

62. (428) Blood salicylate levels of 36 to 40 mg per dl are maintained in therapy of

a. migraine headache.
b. rheumatic fever.
c. strep throat.
d. gout.

63. (429) Pure carbon monoxide gas is

a. arrid.
b. pungent.
c. colorless.
d. sulfurous.

64. (429) Which of the following procedures is not the method ordinarily used for carbon monoxide determination?

a. 30 percent NaOH.
b. Gas chromatography.
c. Palladium chloride.
d. Spectrophotometric-dodium hydrosulfide.

65. (430) Which of the following volatile poisons may be detected by the formation of a color complex with picric acid?

a. Chloroform.
b. Sulfides.
c. Cyanides.
d. Phenols.

66. (430) Which of the following chemicals can be detected from an acid distillate by the formation of insoluble tribromphenol?

a. Trichloroethylene.
b. Caffeine.
c. Cyanide.
d. Phenol.

67. (431) Which of the following drugs is detectable in body fluids?

a. LSD.
b. Heroin.
c. Marijuana.
d. Mescaline.

68. (431) In the rapid test for urinary alkaloids, what other substance besides alkaloids gives a positive test?

a. Globulin.
b. Ketones.
c. Albumin.
d. Mucin.

69. (432) In cases of aircraft accident fatalities, all facilities must send fixed and frozen specimens for toxicological examination to the

a. Armed Forces Institute of Pathology.
b. Epidemiology Division, USAFSAM, Brooks AFB, TX.
c. Malcolm Grow USAF Medical Center, Andrews AFB, Wash, DC.
d. USAF Medical Center, Wright-Patterson, Wright Patterson AFB, OH.

70. (433) If you were to sustain hand burns with sulfuric acid, you would be suffering from an effect classified as

a. cumulative.
b. systemic.
c. remote.
d. local.

71. (433) Compared to adults, the susceptibility of children to poisons is

a. less.
b. the same.
c. greater.
d. variable.

72. (433) A person who is on drugs is not adversely affected by the drugs until an overdose has produced near deadly effects. This type of poisoning may be classified as

a. overdosage.
b. acute poisoning.
c. chronic poisoning.
d. volatile poisoning.

73. (433) An example of chronic poisoning is likely to be found in cases of

a. suicide.
b. overdosage.
c. fuel handling.
d. food handling.

74. (433) Barbiturates may be classified toxicologically as

a. irritants.
b. neurotics.
c. corrosives.
d. volatile poisons.

75. (433) The type of food poisoning caused by the Salmonella group of organisms may be classified as

a. food toxin.
b. food infection.
c. food intoxication.
d. intestinal distress.

STUDENT REQUEST FOR ASSISTANCE

PRIVACY ACT STATEMENT

AUTHORITY: 44 USC 3101. PRINCIPAL PURPOSE(S): To provide student assistance as requested by individual students. ROUTINE USES: This form is shipped with every ECI course package. It is utilized by the student, as needed, to place an inquiry with ECI. DISCLOSURE: Voluntary. The information requested on this form is needed for expeditious handling of the student's need. Failure to provide all information would result in slower action or inability to provide assistance.

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CDC 90411

MEDICAL LABORATORY TECHNICIAN (CHEMISTRY AND URINALYSIS)

(AFSC 90470)

Volume 4

Laboratory Procedures in Urinalysis

Extension Course Institute
Air University

PREPARED BY
SCHOOL OF HEALTH CARE SCIENCES, USAF (ATC)
SHEPPARD AIR FORCE BASE, TEXAS

EXTENSION COURSE INSTITUTE, GUNTER AIR FORCE STATION, ALABAMA

THIS PUBLICATION HAS BEEN REVIEWED AND APPROVED BY COMPETENT PERSONNEL
OF THE PREPARING COMMAND IN ACCORDANCE WITH CURRENT DIRECTIVES
ON DOCTRINE, POLICY, ESSENTIALITY, PROPRIETY, AND QUALITY.

Preface

THIS FINAL volume of CDC 90411 discusses concepts in urinalysis at the 7 level. Chapter 1 begins with a review of proper microscope adjustment and the rather complex area of critical illumination. Other general considerations such as specimen collection and preservation are also reviewed in Chapter 1.

In Chapter 2, we will turn our attention to renal anatomy and physiology which will help us better understand results achieved in the laboratory as discussed in Chapters 3, 4, and 5.

Chapter 3 is a fairly basic presentation of the physical characteristics of urine and their meaning. The two most difficult and meaningful areas of urinalysis are treated in Chapter 4, Microscopic Examination, and in Chapter 5, Chemical Examination. In these last two chapters, some of the practical aspects of urinalysis are presented in depth sufficient to challenge most students. For example, in Chapter 5, you will discover that simple qualitative tests for urinary constituents can be very misleading in certain cases. Reference is made to the sensitivity of the 2-hour pregnancy test versus the 2-minute slide test. A brief discussion on quality control in urinalysis has been introduced.

In studying Volume 4, you should realize that urinalysis is an extremely valuable diagnostic discipline as well as a complicated one. It is not a subject to be dismissed lightly.

Foldout 1 is included at the back of the volume.

Please note that in this volume we are using the singular pronoun *he, his,* and *him* in its generic sense, not its masculine sense. The word to which it refers is person.

If you have questions on the accuracy or currency of the subject matter of this text, or recommendations for its improvement, send them to the School of Health Care Sciences/MST, Sheppard AFB TX 76311. NOTE: Do not use the suggestion program to submit corrections for typographical or other errors.

If you have questions on course enrollment or administration, or on any of ECI's instructional aids (Your Key to Career Development, Behavioral Objective Exercises, Volume Review Exercise, and Course Examination), consult your education officer, training officer, or NCO, as appropriate. If he can't answer your questions, send them to ECI, Gunter AFS AL 36118, preferably on ECI Form 17, Student Request for Assistance.

This volume is valued at 27 hours (9 points).

Material in this volume is technically accurate, adequate, and current as of April 1976.

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5 Chemical Examination 39

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NOTE: In this volume, the subject matter is developed by a series of Learning Objectives. Each of these carries a 3-digit number and is in boldface type. Each sets a learning goal for you. The text that follows the objective gives you the information you need to reach that goal. The exercises following the information give you a check on your achievement. When you complete them, see if your answers match those in the back of this volume. If your response to an exercise is incorrect, review the objective and its text.

General Considerations

WE ARE CONSTANTLY reminded that our purpose within this career field is to support patient care. It is difficult to imagine a single category of laboratory tests which relates more directly and frequently to patient care than an analysis of one sort or another on urine. It is probably safe to say that a routine urinalysis is the most frequently performed laboratory test.

In beginning this study of urinalysis, let us observe the danger signs that confront us. First, urinalysis is *not* a simple and dull subject, though it is often considered as such. Second, it is *not* a monotonous chore which, at best, does the patient no harm. Finally, the urinalysis section of a laboratory *is* a vital and vibrant area of direct concern to the patient and to the physician. If you doubt this, just ask any practicing physician how he evaluates the importance of this examination. The number of urine tests you are likely to perform in your career will far exceed the number of more complicated and involved determinations of other kinds.

1-1. Review of Microscopy

It is impossible to perform an accurate urine microscopic examination without an adequate optical and mechanical system. In this section we will discuss proper use and care of the microscope at the 90470 level. The understanding of an acceptable method of adjustment and illumination is more rewarding than trial and error technique.

600. Indicate whether given statements reflect the correct procedures for preliminary adjustment and focusing of the lamp filament of the microscope.

Preliminary Adjustment of the Microscope. The modern microscope is a precision optical instrument which, like all fine instruments, requires care in its use. It also requires knowledge of the instrument on the part of the user. The relationship of the microscope lamp to the microscope is critical, because this relationship governs the intensity of the illumination which is available, as well as the quality of the final image which the eye sees. Since microscopes are seldom in constant use, it is desirable to maintain the relative position of lamp to microscope by means of a fixed mounting. This avoids the necessity of reestablishing the lamp-microscope relationship each time the scope is used. A microscope mounting board may be fabricated locally.

Preparatory to setting up the illumination system, review the components of the microscope illustrated in figure 1-1. After placing a slide on the microscope stage, swing the 10X objective into place. Adjust the microscope mirror so that the slide is illuminated. Move the condenser to its uppermost position (see substage control knob, fig. 1-1). Using the coarse focus adjustment of the microscope, obtain an image of the specimen on the slide. Next, turn the ocular focusing control to zero. Adjust the interpupillary distance control so that the lateral spread of the two eyepieces is comfortable to the eyes and only one image is seen. While doing this you will see that the image of each eyepiece fuses, to appear as a singular circular image. Note for future reference the scale setting which is on the front of the microscope between the eye tubes.

With the objective fine focus adjustment, obtain a critical focus of the specimen on the slide, looking only through the eyepiece that does not have the ocular focusing control. For convenience, a piece of opaque paper may be placed over the eyepiece of the tube with the ocular focusing control. After obtaining a critical focus, switch the paper to the nonfocusing control eyepiece so that the eyepiece is obstructed. Do not further touch the objective fine focus adjustment. Using the knurled ring of the ocular focusing control, bring the image into fine focus for your eye. The microscope will then be adjusted to accommodate for small differences in refraction of your two eyes. At this point note the numerical setting on the ocular focusing control ring for future reference.

A person having astigmatism necessitating constant use of glasses may wish to use a special set of oculars

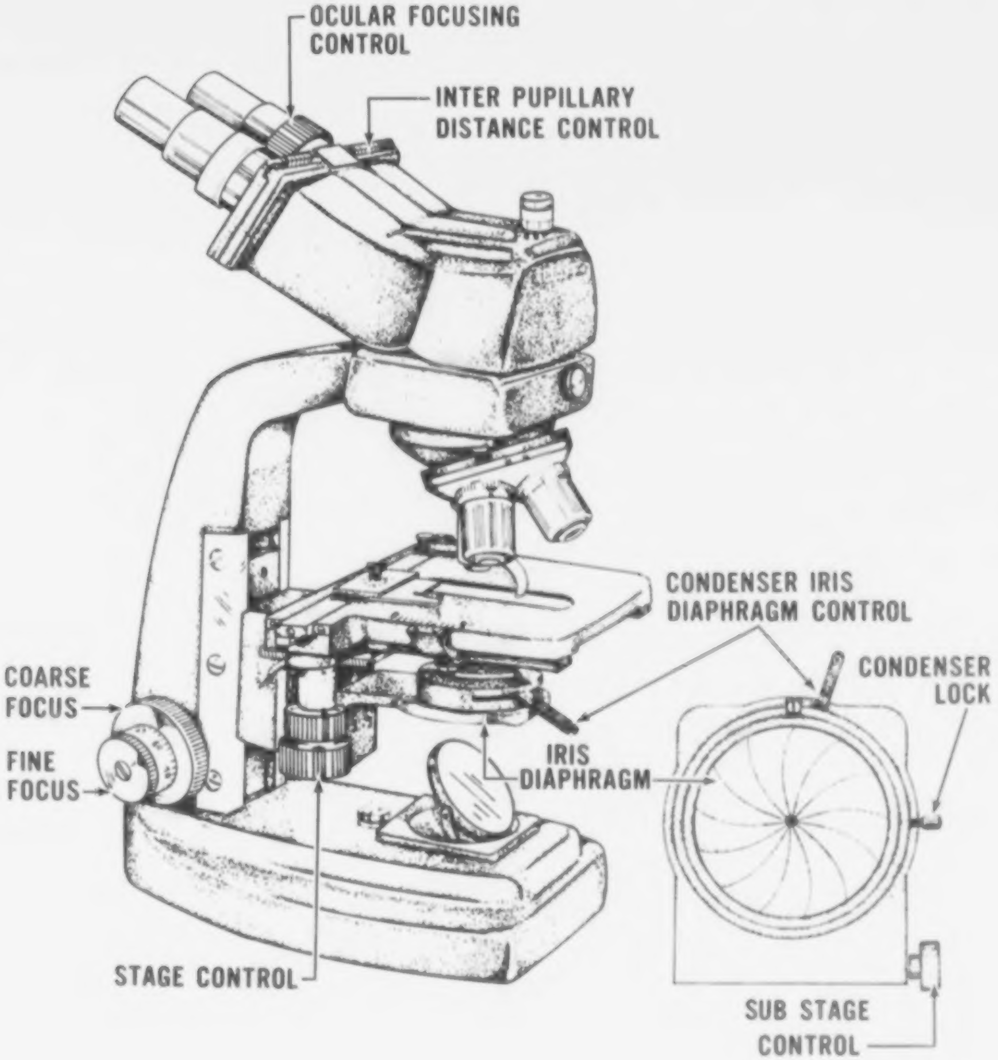
OCULAR FOCUSING CONTROL
INTER PUPILLARY DISTANCE CONTROL
CONDENSER IRIS DIAPHRAGM CONTROL
CONDENSER LOCK
COARSE FOCUS
FINE FOCUS
IRIS DIAPHRAGM
STAGE CONTROL
SUB STAGE CONTROL

Figure 1-1. Clinical laboratory microscope.

designed for use with spectacles; however, these are not available through regular supply channels in the Air Force. The proper name for the system of illumination which will now be described is "Kohler of illumination."

Focusing the Lamp Filament. Before continuing, study the components labeled in figure 1-2. These will be referred to in the description that follows. Place a piece of filter paper or a white 3'' x 5'' card over the mirror of the microscope as shown in figure 1-2. Be sure that the flat side of the mirror is facing forward. (The curved side of the mirror is never to be used with an artificial light source.) Remove the filter holder from the lamp. Close the iris diaphragm of the lamp as far as it will go. Do not force this or any other control. Align the lamp so that it is directly in front of the microscope and is at a distance of approximately 8 to 12 inches from the microscope. By means of the knurled knob at the back of the lamp base, shown in figure 1-2, adjust the vertical position of the lamp so that the light falls on the card or filter paper covering of the microscope mirror. With the filament focus control, move the lamp tube back and forth until the filaments of the lamp bulb are sharply focused on the card or filter paper shown in figure 1-2. Adjust the horizontal and vertical directions of the lamp so that the image of the filaments is centered on the mirror.

Focusing the lamp filaments on the mirror is a convenient method of establishing the illumination. However, an even more accurate method is to take a second flat mirror and position it adjacent to the microscope mirror so that one can see the underside of the condenser iris diaphragm. The condenser iris diaphragm is closed so that its opening is but a pinpoint. Adjust the lamp filament focus controls so that the image of the lamp filaments is in focus and centered on the underside of the condenser iris diaphragm.

Exercises (600):

You are preparing to set up the illumination system of the microscope and to focus the lamp filament. Indicate whether the procedure to follow is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. After placing the slide on the microscope stage, swing the 10X objective into place.

T F 2. A critical focus of the specimen on the slide must be obtained with the fine focus adjustment looking only through the eyepiece which does not have the ocular focus control.

T F 3. Using the knurled ring of the coarse focusing control, bring the image into fine focus for your eyes.

T F 4. Preliminary microscope adjustment allows for small refractive differences of your eyes.

T F 5. The curved side of the mirror is to be used with an artificial light source.

T F 6. The filament control will move the lamp up and down until the filaments of the lamp bulb are sharply focused on the card or filter paper.

T F 7. Focusing the lamp filaments on the mirror is the most accurate method of establishing the illumination.

601. Indicate whether given statements correctly reflect the procedures for focusing the condenser.

Focusing the Condenser. Check the microscope image of the slide object. Be sure this is still in focus. Now be sure that the condenser iris diaphragm is closed. With both the condenser iris diaphragm and the lamp iris diaphragm closed, move the microscope condenser up and down until a sharply defined image of the lamp iris diaphragm is obtained in the field of view. Once again the image of the object on the slide should be in sharp focus. Adjust the microscope mirror so that the image of the lamp iris diaphragm is centered in the middle of the field of vision. Open the iris diaphragm of the lamp until the full field of vision seen through the microscope eyepieces is fully illuminated. No further adjustment to the lamp or microscope condenser is required.

Some microscopes may acquire an additional auxiliary lens, which is of the swing-out variety, beneath the condenser before full field illumination is obtained. This depends on the condenser attached to the microscope. In such instances the auxiliary lens is kept out of the optical pathway until the lamp adjustments and condenser adjustments are completed.

Replace the filter holder on the lamp, being careful not to disturb the position or adjustment of the lamp. You will note that a filter holder has a blue-tinted color correction filter, a white diffusion disc, and a snapring to hold the tube components, color filter, and diffusion disc in place. There is room for additional filters in the external rack of the filter holder.

Once the illuminating lamp and the microscope condenser have been focused, the only other adjustment which must be made is that for the condenser iris diaphragm. This iris diaphragm is not intended to control the level of illumination seen in the microscope. Instead, it is intended to serve as a part of the optical system which is responsible for resolution of the image. There is only one proper iris diaphragm control setting for each of the objectives on the microscope. In order to properly adjust the iris diaphragm, carry out the steps outlined in the following paragraphs.

Exercises (601):

Indicate whether the following statements are true (T) or false (F). If you indicate "false," explain your answer.

T F 1. The initial step in focusing the condenser is to make sure that the lamp iris diaphragm is open and the condenser diaphragm is closed.

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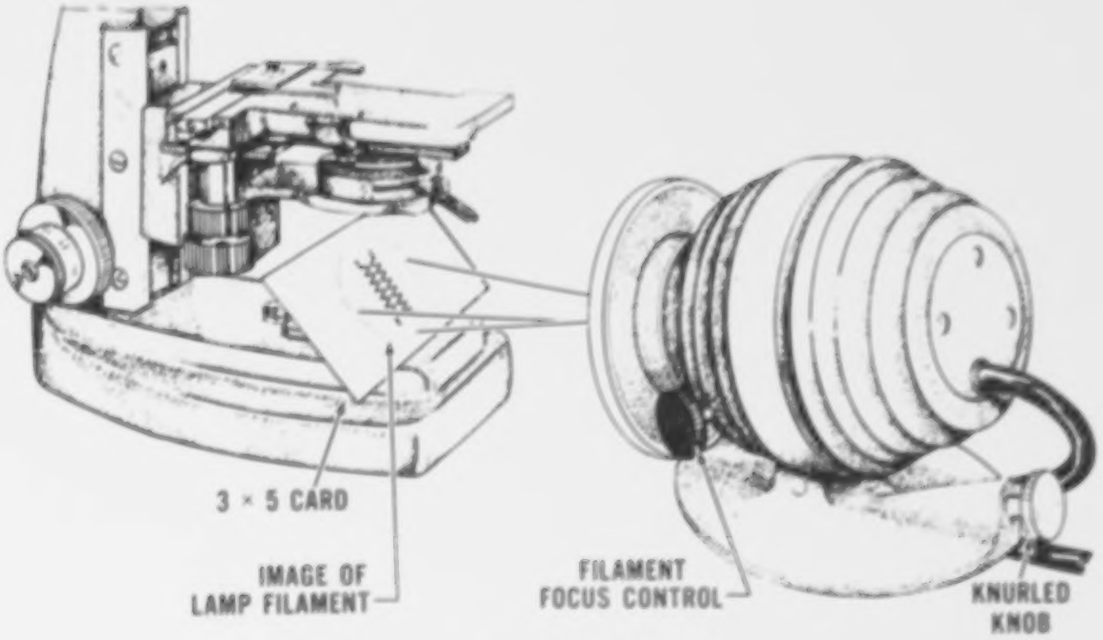


Figure 1-2. Microscope lamp illumination.

T F 2. Auxiliary lens is kept out of the optical pathway until the lamp and condenser adjustments are completed.

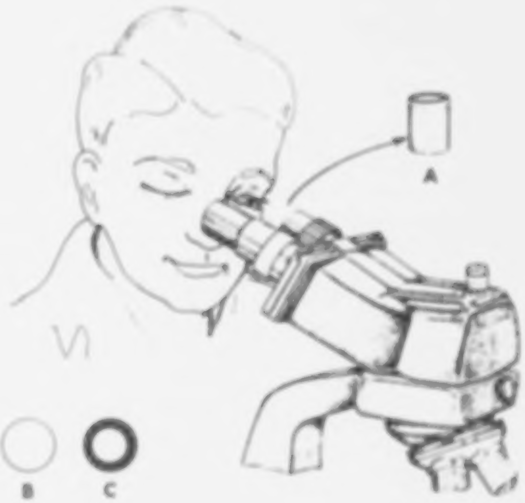


Figure 1-3. Adjusting the condenser diaphragm.

T F 3. After the illuminating lamp and the condenser have been focused, the only other adjustment to be made is that for the condenser iris diaphragm.

T F 4. The condenser iris diaphragm is intended to control the level of illumination.

602. Point out the procedures for adjusting the condenser iris diaphragm, controlling the illumination, and minor maintenance of the microscope.

Adjusting the Condenser Iris Diaphragm. In order to adjust the condenser iris diaphragm, it is necessary to open it fully. Remove one of the oculars (eyepieces of the microscope), as shown in figure 1-3,A. If you look down the eye tube, you will see that the back lens of the objective is fully filled with light. At this point if you close the iris diaphragm of the condenser to its fullest closed position, you will see that there is but a pinpoint of light visible at the back lens of the objective. Once again fully open the iris diaphragm of the condenser (fig. 1-3,B) and then slowly close it until the diameter of the back lens' illumination is slowly relaxed. Continue to close the iris until approximately two-thirds to three-quarters of the diameter of the back lens is fully illuminated. This leaves the outermost one-third to one-fourth of the back lens of the objective nonilluminated as shown in figure 1-3,C. Replace the ocular (eyepiece). If you follow the preceding steps, your microscope will be properly adjusted for Kohler illumination which will produce the finest resolution the microscope is capable of offering.

When your microscope has a revolving nosepiece and you are using more than one objective, you will find that it becomes necessary to slightly readjust the entire series of adjustments which we have previously described. For this reason it is advisable to make your first alignment using the 10X objective. Then go to your oil immersion objective and make the fine adjustments necessary to produce a sharply focused centered image of the iris diaphragm of the lamp. Also, properly adjust the iris diaphragm of the condenser. With the lamp focused, and mirror condenser adjustments aligned for the oil immersion lens, you should not further adjust the lamp or condenser for the objectives except for the condenser iris diaphragm. Each objective will require a slightly different setting of the condenser iris diaphragm to produce the desired reduction in illumination of the back lens of the objective.

In a similar manner, the extent to which the lamp iris diaphragm must be open to produce a fully illuminated field will vary according to the objective used. For convenience, allow this to remain at the setting required for the lowest power objective being used (usually 10X).

Never attempt to use objectives having a high numerical aperture (N.A.) value with a condenser having a lower value. Ask your pathologist, laboratory officer, or supervisor to check your microscope and its optics in this regard. However, this loss in resolution is relatively slight in comparison with that obtained when the illumination is improperly designed for the oil immersion objective.

Controlling Illumination. The intensity of illumination should be controlled with neutral density filters. Do not attempt to control the intensity of light as your eyes see it by opening or by using the condenser iris. This is incorrect. Likewise, you cannot control the intensity of illumination with the lamp iris. The lamp iris is used only to cut out extraneous light once it has been opened sufficiently to produce a full field of illumination. The intensity of illumination may be controlled by regulating the lamp voltage using a lamp equipped with a rheostat, in which case suitable color correction filters to produce a white or blue-white light are required. If the voltage cannot be controlled, you should use neutral density filters to reduce the amount of light received. Within limits, the intensity of illumination may also be controlled by placing the illuminating lamp closer to, or farther from, the microscope. However, this will require readjustment of the lamp focus. Once you have properly established the relationship of the microscope lamp to the microscope and adjusted the lamp and condenser focus, then these relationships can be maintained by locking the lamp to a lamp board if your scope has been mounted as suggested.

Microscopes with built-in illumination systems should have separate controls for determining the area of visual field that is illuminated and for determining the size of the aperture. These controls are used in the same manner as that described for an external lamp.

Minor Maintenance. Before concluding our discussion of the microscope, let us briefly review some points of good technique which should already be thoroughly familiar to you. A monocular microscope should be used with both eyes open. Lenses must not be touched except with lens paper. Mirrors, slides, cover glasses, and other microscope equipment may be cleaned with lint-free tissue or with a soft cloth. Do not insert or remove a slide under high power. Most microscopes are parfocal, which means that the viewer can switch from one power to another with minimal refocusing. Always wipe the oil from the oil immersion objective when you have finished using the microscope. Switch to lower power when finished rather than leaving the oil immersion objective in a viewing position. Last of all, keep the microscope covered when it is not in use.

Exercises (602):

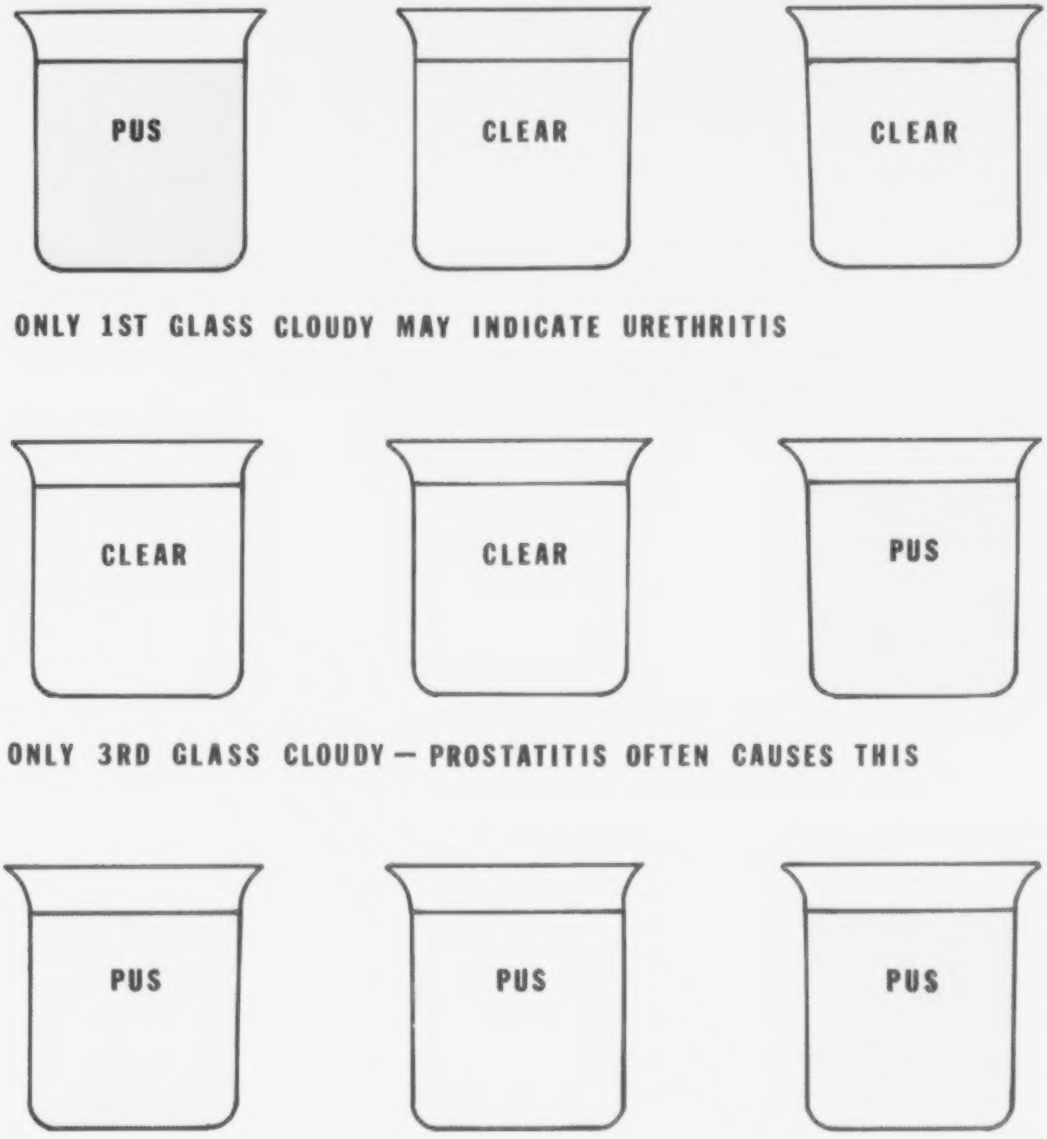
1. What's your first step before you look down the eye tube to see the back of the objective when adjusting the condenser iris diaphragm?

2. How should the iris diaphragm be adjusted before replacing the ocular?

3. Why would each object require a slightly different setting of the condenser iris diaphragm?

4. How should you control the intensity of illumination once your microscope and lamp system have been established for Kohler illumination?

5. What material should be used to clean the microscope mirrors, slides, cover glasses, and other related equipment?


PUS
CLEAR
CLEAR
CLEAR
CLEAR
PUS
PUS
PUS
PUS

ONLY 1ST GLASS CLOUDY MAY INDICATE URETHRITIS

ONLY 3RD GLASS CLOUDY — PROSTATITIS OFTEN CAUSES THIS

ALL THREE GLASSES CLOUDY — BLADDER AND/OR RENAL INFECTION SUGGESTED

Figure 1-4. Observation of the three-glass urine specimen test.

1-2. Collecting and Preserving Specimens

As simple as it is to obtain a urine specimen, reliability of results depends upon reasonable care and precautions associated with initial processing in the laboratory.

603. Identify responsibilities of personnel in the collection of urine specimens and indicate whether given statements correctly reflect the procedures for collecting specimens.

Few technicians would treat "more valuable" specimens like cerebrospinal fluid as lightly as they do urine specimens. Quantitative determinations on urine are of clinical value only when performed on properly collected and prepared 24-hour specimens. The accurate collection of such specimens from ward patients and most particularly from outpatients has for many years presented certain technical and esthetic problems. Ward personnel are extremely reluctant to store urine specimens during the collection period in refrigerators containing drugs or foods. This is equally true of specimens collected in the home. However, the bottle must be kept in the refrigerator for certain types of urine chemical analyses. With some care, one can assure that the outside of the bottle is clean and free from urine, which might contaminate other contents of the refrigerator. If a preservative or other additive has been placed in the bottle, it is essential that the contents be gently but thoroughly mixed after each addition. Let us consider some of the common areas of concern dealing primarily with handling of the specimens.

Collecting the Specimen. How a specimen is to be collected is determined by the physician with technical assistance from laboratory personnel. However, keep in mind that the responsibility for the accuracy of urine collections lies first with laboratory personnel who must be familiar with the necessary preservatives or other additives required and who supply properly prepared collection containers to the wards or directly to outpatients. The second responsibility is that of the patients. Without this, accurate collections are impossible. This is one of the transitional areas in which neither the clinician nor the laboratory always knows precisely how each specimen was collected.

The nursing service of a hospital can often be of tremendous value in assuring that a specimen is of the type the physician intended and what the laboratory thinks it is. To cite a few examples, an early morning specimen differs in clinical significance from one collected later. One cannot always assume that a sample which arrives in the laboratory in the morning was actually collected in the morning. It is also of concern that specimens be fresh or properly preserved. Hyaline casts, especially, will dissolve in neutral urine of low specific gravity or in any urine that is alkaline. Urine which has been standing becomes alkaline due to bacterial and chemical activity. In many cases, even if laboratory examination of the specimen is prompt, the urine may have been unsatisfactory when it arrived. It is your responsibility as a technician to evaluate specimens for technical acceptability.

Although a request form has a block for date and time of collection, the entry one finds rarely has any relationship to the time the patient voided. This is not so much a case of negligence as a practical problem. For outpatients, the requirement almost necessitates an entry by the patient himself, and this is more than the most optimistic technician can consistently expect. An even greater error is introduced by incomplete specimens. Not infrequently, the patient is improperly instructed with regard to the necessity of collecting *all* voidings within the 24-hour period. The patient often forgets to void into the container, is too preoccupied to obtain his container from the storage refrigerator, or else is too shy to bring the specimen to the collection bottle. Because of these personal problems and environmental objections, such as may be encountered in a school, in an office, or on the flight line, a mutually satisfactory time can be determined. You, as the technician, should decide from the physician's request, the situation of the patient and laboratory scheduling the optimum time for the collection. If the physician requests a specific period of time for collection, of course, the orders should be followed to the letter. Parents may require special assistance in securing urine specimens from small children. You may provide plastic collecting containers designed for pediatric patients and give instructions in container use.

In reality, the best practice is for collection to be made in or near the laboratory. For inpatients, there are also problem areas. Specimens transferred from other receptacles may be contaminated. But perhaps more common, specimens sometimes remain on the ward until personnel are free to pick them up or send them to the laboratory. Laboratory slips are frequently completed ahead of time; therefore, as with outpatients, these forms do not always reflect accurate entries regarding time of collection. This does not imply a criticism of the nursing services. It is merely a fact which must be dealt with if the result is to be meaningful.

Instructions for Collection. Collecting a specimen sometimes requires special instructions. It is assumed you are thoroughly familiar with the terms *random specimen, clean catch, midstream, two-glass,* and *three-glass.* Figure 1-4 indicates how laboratory observation of three-glass urine specimens could be interpreted by the physician. Needless to say, the diagnosis requires more than laboratory observation, but this does illustrate the value of three-glass specimens.

In advising outpatients on collection techniques, there is a tendency for the technician to assume too much or in some cases to be purposefully vague, perhaps to avoid embarrassment. A patient may not have the slightest idea what a midstream specimen is or how to collect it. This also applies to other special instructions. Instructions should be given in an explicit and professionally tactful manner. Sometimes the special handling of a specimen involves more chance for error than a slightly more casual approach. Consider, for instance what is necessary to obtain a clean-catch specimen. A clean-catch procedure which requires elaborate instructions and the use of cleansing agents is less desirable than a simpler method. A mild antiseptic must be used carefully prior to collecting the urine specimen. A more potent cleansing agent could contaminate the specimen. Some of these are potent antibacterial agents and could render the urine sterile before it is cultured. The subject of urine cultures will be covered in greater detail in a volume of the microbiology course. However, it is mentioned here that a simple procedure is often better than an involved one by the very reason of its simplicity.

It is expected that all of the usual laboratory precautions will be observed, including the use of clean or sterile containers. Rinsing a bottle and using it over again and again is *not* good technique. At least thorough washing with detergent and hot water is required. Contaminants, and especially molds which adhere to the container, can confuse a microscopic analysis. It might be argued that bottles should be sterilized as a good hospital practice to avoid possible contamination to individuals. Urine bottles from a contagious case (for example, hepatitis) must certainly be sterilized. Mere rinsing is a widespread practice but hardly one to be recommended. Ordinary detergents do *not* inactivate viruses or even eradicate bacteria. Finally, cracked or chipped bottles should be discarded. Saving faulty glassware is false economy and downright dangerous in any laboratory situation. The use of broken glassware is inexcusable. If you work in a laboratory, it is suggested that you discard all broken or chipped glassware that cannot be economically repaired. This does not apply just to urinalysis. Glass items that can be repaired must not be used in the interim. If possible, substitute plastic or other nonbreakable items as a matter of economy and laboratory safety. Disposable plastic containers with lids are available in several standard sizes and are preferred for routine screening urinalysis.

Exercises (603):

In exercises 1 through 5, match each duty in collection of urine specimens in column A with the personnel responsible in column B by placing the letter of the column B item in the appropriate space in column A.

Column A	*Column B*
___ 1. Accuracy of urine collections.	a. Responsibility of nursing staff.
___ 2. Type of specimen collected.	b. Responsibility of patient.
___ 3. Method of specimen collection.	c. Responsibility of laboratory personnel.
___ 4. Acceptability of specimens of inpatients.	d. Responsibility of physician.
___ 5. Advising outpatients on collection techniques.	

In exercises 6 through 15, indicate whether each statement about the handling of urine specimens in the laboratory is true or false. If you indicate "false," explain your answer.

T F 6. Quantitative determinations can be made on any type of specimen.

T F 7. Specimens should never be stored in refrigerators with drugs and food.

T F 8. Urine that has been allowed to stand becomes alkaline due to bacterial and chemical activity, and any hyaline casts present will dissolve.

T F 9. The time of collection entered on the request form is usually the time the patient voided.

T F 10. To assure that the specimen is of the proper type and that it is fresh or properly preserved, the best place for collection is in or near the laboratory.

T F 11. Use a mild antiseptic to preserve urine specimens.

T F 12. Rinse the container before using it again.

T F 13. Collection bottles should be sterilized before reuse.

T F 14. An ordinary detergent will inactivate viruses and eradicate bacteria.

T F 15. Broken, cracked, or chipped glassware should be discarded.

604. Indicate whether given statements correctly reflect the importance of urine preservation and the advantages and disadvantages of the various preservatives used.

Preservation of Urine. There is no substitute for a fresh urine specimen, and in all cases analysis should be accomplished as soon as possible. On occasion, however, it is not possible to analyze the sample immediately (for example, 24-hour specimens). A delay in analysis leads to degeneration of the formed elements and decomposition of chemical constituents. An example is the breakdown of urea into ammonia by the action of contaminating micro-organisms.

Deterioration may be inhibited by the use of some form of preservation. Always keep specimens refrigerated when they cannot be promptly analyzed. It is important that specimens warm to room temperature before analysis is performed to reduce turbidity and enable the technician to make accurate quantitative measurements, including specific gravity determinations.

Chemical Preservatives. A number of chemical preservatives can be utilized with varying degrees of effectiveness. The following are recommended for use *in conjunction with refrigeration.*

Toluene. Toluene (sufficient quantity added to form a thin layer over the specimen) is a very satisfactory inhibiting agent used when chemical analysis is required because the reagent does not interfere with most chemical tests. Anaerobic bacteria which might be present in the sample continue to multiply, however. It is necessary either to remove the toluene before measuring portions of the specimens or to pipette from below the surface. Toluene is not recommended for collection of urine specimens for steroid determinations. Free steroids are extracted into the toluene, while ketogenic steroids and other water-soluble conjugates will remain in the urine. Unless care is taken to mix the specimen thoroughly, part of the free steroids will be lost and low values will be obtained.

Formalin. Formalin (about 4 drops/100 ml urine) is the best preservative for formed elements. It interferes with some chemical analyses, and should not be used when sugar concentration is to be determined by reduction methods. Formalin also inhibits the Obermayer test for indican. Preservative tablets that produce formaldehyde such as Urokeep and Cargille Urinary Preservative tablets are available and are much more convenient to use than liquid formaldehyde. These tablets do not interfere with the usual chemical and microscopic examination.

Thymol. Thymol may be added to the extent of a few small crystals for every 100 ml of urine. It gives a false positive albumin test, however, and may interfere with tests for bile.

Special preservatives. Special preservatives include hydrochloric acid for vanilmandelic acid (VMA), serotonin, and other analyses discussed in Volume 3 of this course. Boric acid and chloroform are other preservatives used. Sodium fluoride may be used to preserve glucose in urine. For many purposes, it is possible to keep the urine refrigerated without preservatives added. When analyses for total nitrogen, amino acids, and delta-amino-levulenic acid are to be made, the urine must be acidified with a strong mineral acid; for example, concentrated HC1 to pH 3.

If a chemical preservative has been added to a urine specimen, the type and amount must be indicated on the label of the collection bottle. Always follow instructions of the reference laboratory when you preserve specimens for shipment.

In handling 24-hour specimens, the laboratory should insure that the entire specimen is well mixed. Measure the total volume in a 1- or 2-liter graduated cylinder to the nearest 5 ml. Whenever possible, collection periods should *not* begin on Friday or Saturday, to avoid having to store specimens over the weekend. If this is unavoidable, store the entire specimen in the refrigerator or freezer until shipped.

Exercises (604):

Indicate whether each of the following statements concerning the preservation of urine specimens is true or false. If you indicate "false," explain your answer.

T F 1. Formed elements degenerate and chemical constituents break down if specimens are allowed to stand.

T F 2. Chemical preservatives can be used in conjunction with refrigeration.

T F 3. Toluene may be used as a preservative in steroid determinations.

T F 4. Formalin is the best preservative for formed elements.

T F 5. Thymol is the best preservative in tests for albumin or bile.

T F 6. No preservative should be used when tests for amino acids or delta-amino-levulenic acid are to be made.

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Renal Functions

ONE REASON THE clinical laboratory performs so many urinalysis procedures is the vital importance of the kidneys. There is an ever-increasing interest in the role of the kidneys in maintaining electrolyte and fluid balance and in their relationship to certain diseases. The composition of the blood and the internal structure are determined not only by what is ingested by mouth but also by what the kidneys retain.

The kidneys maintain this internal environment by filtering blood, by selective reabsorption, and by the secretion of foreign substances from the blood.

A variety of urine tests have been established which aid the physician in diagnosing and treating many disorders. To understand the importance and meaning of urinalysis, you should have some knowledge of the kidney itself. In this chapter you will become familiar not only with the structure of the kidney and with the physiology of the normal kidney but also with the correlation between certain diseases and the functions of the kidney.

As you gain insight into the application and importance of urinalysis tests, you will be less inclined to draw conclusions about the clinical condition of the patient. If you appreciate the involved relationships which exist between a laboratory test and the diagnosis of a disease, you will perform the test with greater care and interest. You will realize that a "normal" urine result can be as valuable to the physician as an "abnormal" report. It is important that you know the clinical impact and importance of your work, and at the same time refrain from the role of diagnostician. Always think in terms of your responsibilities as a clinical laboratory technician and the welfare of the patient.

2-1. Anatomy of the Renal System

If an engineer attempted to design a filtration system, his design could hardly be more successful than the kidney. The kidney is a remarkable organ, quite complicated in structure and function. As William Bowman, an English physiologist (1816-1892), stated in 1842, "It would indeed be difficult to conceive a disposition of parts more calculated to favor the escape of water from the body. . . ." Of course, the functions of the kidney are vastly more complicated than Bowman implied or realized. In this section you will learn the basic structures of the kidney by studying this "disposition of parts."

605. Name the four parts of the nephron unit, match renal structures with their appropriate descriptive statements, and identify specific parts from a given illustration of the kidney.

The Kidney. Nearly everyone is generally familiar with the appearance of a kidney. The kidneys are bean-shaped organs situated in the posterior part of the abdomen on either side of the vertebral column between the 12th thoracic and the 3d lumbar vertebrae. Because of the position of the liver, the right kidney is slightly lower than the left. The kidneys are not rigidly attached but move slightly with respiration. Each kidney is enclosed by a thin, translucent capsule which is penetrated by a number of blood vessels. There is an indentation known as the hilum which expands into the renal sinus. The kidney consists of several more or less distinct lobes which become less apparent with age. If you slice a kidney open, you will observe the lobed appearance in the central portion or medulla. The peripheral portion of a kidney is known as the cortex, as shown in figure 2-1. The appearance of the cortex varies considerably with pathological conditions. Normally, the cortex of a kidney appears granular with ray-like structures known as medullary rays. These rays are visible in the cortex, as illustrated in figure 2-1, and not in the medulla as the name would imply. The medullary ray is composed of branched collecting tubules and limbs of the loop of Henle. The lower portion of the loop of Henle extends into the medulla.

Structures Related to the Kidneys. Structures directly related to the kidney, but not a part of the kidney, include the extrarenal blood vessels, ureters, bladder, and urethra. Blood is supplied to the kidneys by way of the abdominal aorta and returns to the heart by way of the inferior vena cava. Two renal arteries lead from the abdominal aorta to supply blood to the kidneys. There are two ureters, one leading from each kidney to the bladder. They are usually 12 to 15 inches long and carry urine from the kidneys to the bladder

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with the aid of muscular action. From the bladder, urine is passed from the body through the urethra.

The Nephron Unit. The functional unit of a kidney is the nephron, which can be seen in figure 2-2,A. The nephrons average 50 to 55 millimeters in length and are situated in the cortex close to the medulla. There are approximately 1,300,000 to 2,000,000 nephrons in each kidney. If all of the tubules of both kidneys were stretched out in one direction they would extend for approximately 75 miles! Blood is supplied to each nephron unit by means of an afferent arteriole leading from branches or endings of the two renal arteries. One afferent arteriole may supply more than one nephron. Blood is carried from the unit by means of an efferent arteriole through a capillary network into branches of the renal vein for return to the heart via the inferior vena cava. The nephron unit consists of four parts: the glomerulus; the proximal convoluted tubule; the loop of Henle; and the distal convoluted tubule. The glomerulus is also referred to as the renal corpuscle. A glomerulus averages from 150 to 250 microns in diameter, and there are about 1,500,000 glomeruli in each kidney as previously mentioned.

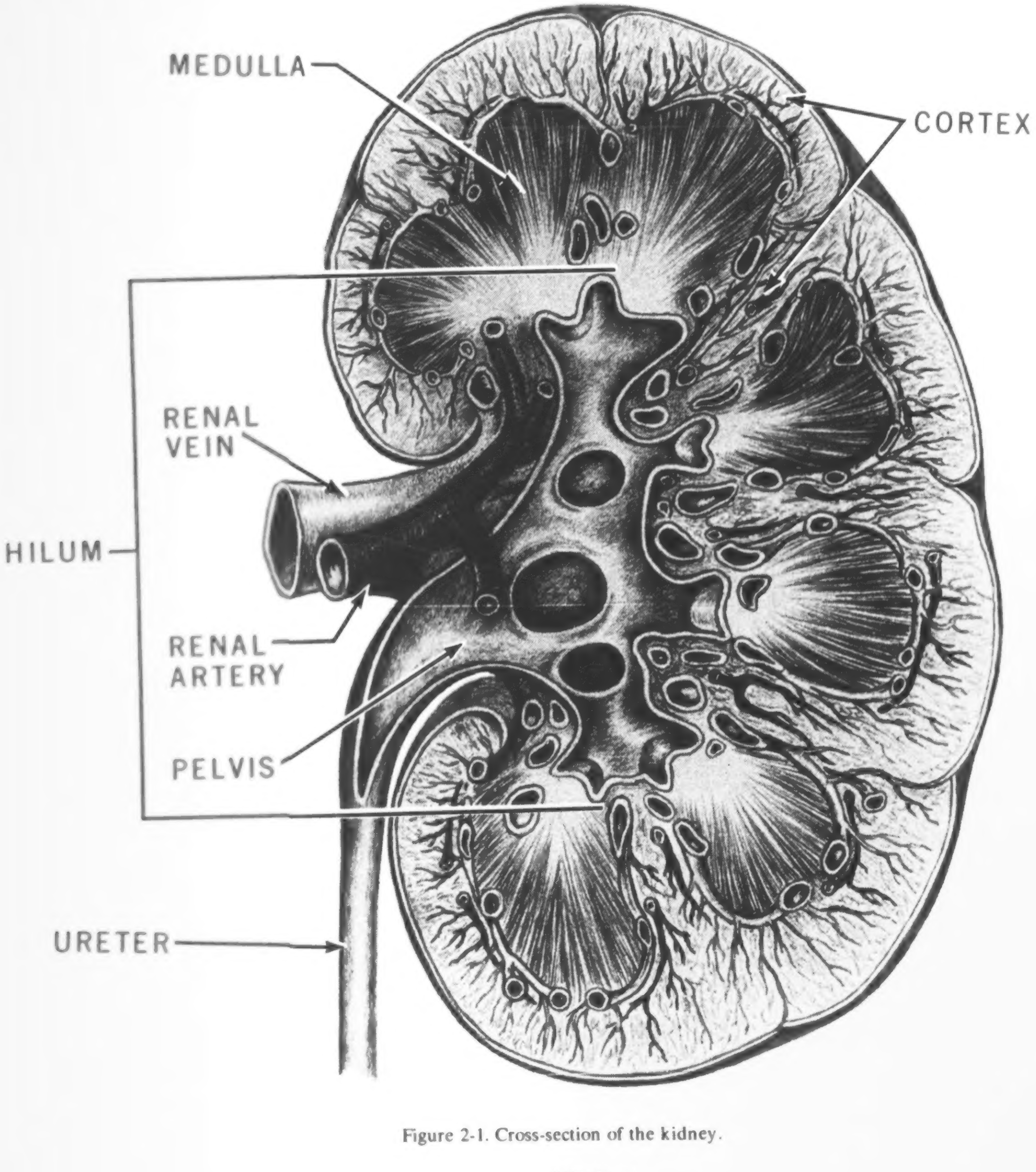

Figure 2-1. Cross-section of the kidney.

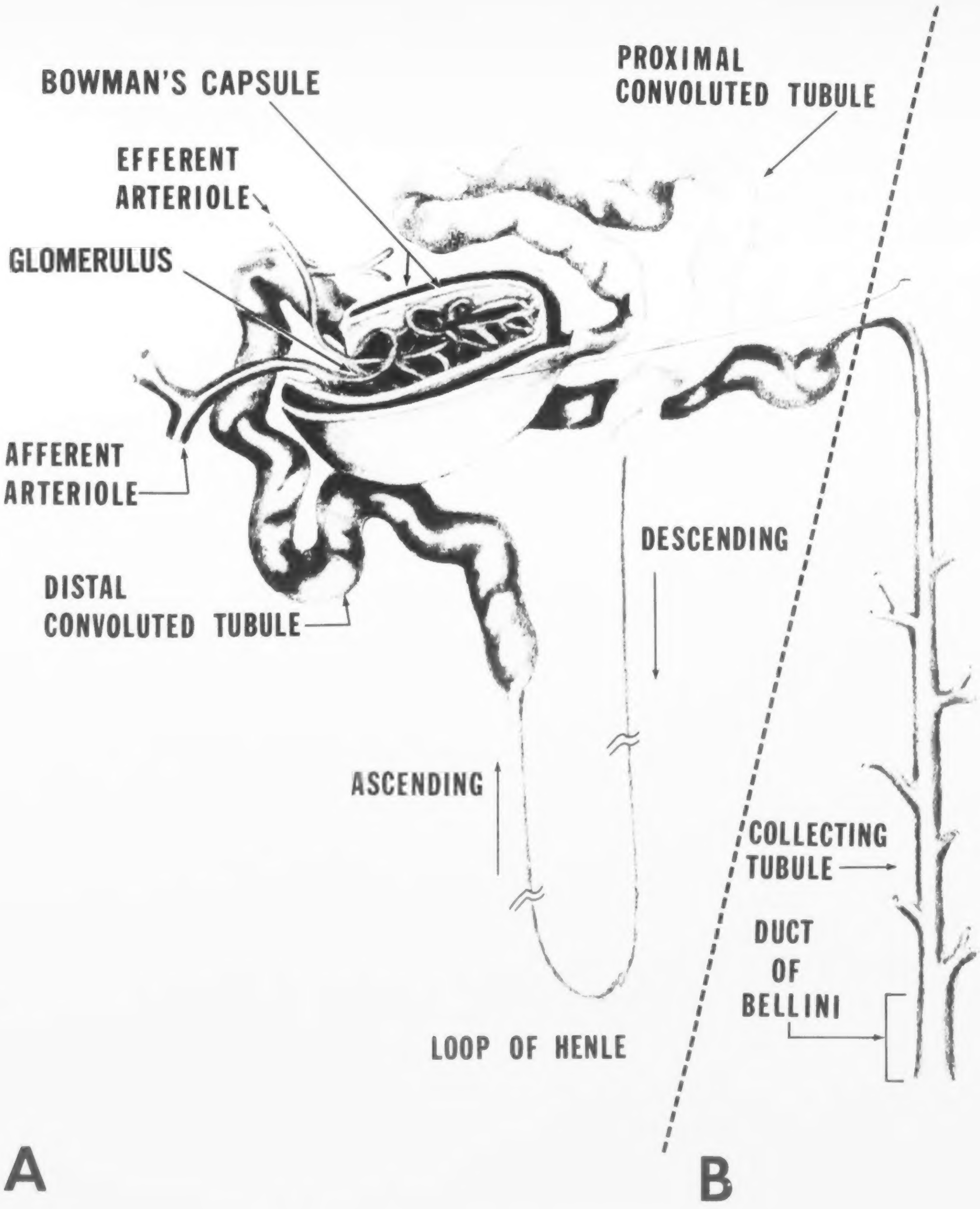
BOWMAN'S CAPSULE
PROXIMAL CONVOLUTED TUBULE
EFFERENT ARTERIOLE
GLOMERULUS
AFFERENT ARTERIOLE
DESCENDING
DISTAL CONVOLUTED TUBULE
ASCENDING
COLLECTING TUBULE
DUCT OF BELLINI
LOOP OF HENLE
A
B

Figure 2-2. The nephron unit and collecting tubule.

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Each glomerulus consists of Bowman's capsule and Malpighian tuft. Bowman's capsule is a double-walled epithelial sac which surrounds the tuft and receives glomerular filtrate. The Malpighian tuft consists of a network of capillaries. This capillary tuft is derived from the afferent arteriole which enters the capsule. The afferent arteriole divides into two to ten primary branches, which in turn subdivide into fifty capillary loops. These capillaries join to form the efferent arteriole which leaves the Bowman's capsule adjacent to the afferent arteriole. As a part of the tuft, the afferent arteriole and efferent arteriole serve to present evidence of glandular activity and a means of regulating glomerular blood pressure, respectively. The tuft may be identified as the glomerulus, as shown in figure 2-2. The proximal convoluted tubules are about 14 mm in length and are lined with structurally similar cells. (These cells do not all have the same function, however.) The cells of a proximal tubule are quite different from those lining the descending limb of the loop of Henle. The ascending limb of the loop, which leads into a distal convoluted tubule, is somewhat larger than the descending limb. The distal tubule is approximately 5 mm in length and 20 to 50μ in diameter. The cells lining the distal tubules are quite characteristic of that portion of the tubules. Although not part of the nephron unit, a system of collecting tubules begins in the cortex and eventually unites with other tubules in large collecting tubules labeled *ducts of Bellini* (see fig. 2-2,B). Cells lining the collecting tubules are unlike those lining secretory tubules. Histopathologists are very much aware of pathological changes which occur in cells of the tubules under various conditions. Tubular cells have recently been studied in detail with the electron microscope. It has been demonstrated that many biochemical processes take place in cells of the tubules, partly by relating the presence of structures shown with the electron microscope to their known activities in other cells. This is an example of how knowledge of structure can lead to an understanding of function. Obviously morphology alone does not supply all of the information desired. With this in mind, we will now turn our attention from structure to function.

Exercises (605):

1. List the four parts of a nephron unit.

In exercises 2 through 10, match each structure of the renal system in column B with its correct descriptive statement in column A by writing the letter of the column B item in the appropriate space in column A.

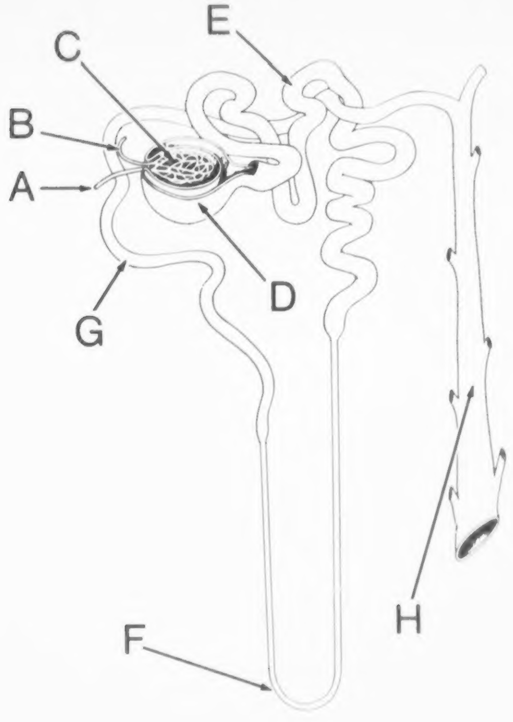
Column A

___ 2. Peripheral portion of kidney.
___ 3. Central portion of kidney.
___ 4. Indentation on kidney.
___ 5. Ray-like structures composed of branched collecting tubules and limbs of the loop of Henle.
___ 6. Structure that supplies blood to nephron unit.
___ 7. Structure that carries blood from the nephron unit.
___ 8. Another name for the glomerulus.
___ 9. Part of the glomerulus that projects into the uriniferous tubule.
___ 10. Large collecting tubules.

Column B

a. Malpighian tuft.
b. Afferent arteriole.
c. Hilum.
d. Ducts of Bellini.
e. Cortex.
f. Renal corpuscle.
g. Medulla.
h. Medullary rays.
i. Efferent arteriole.

2. Name four mechanisms by which the kidneys maintain the acid-base or electrolyte balance of the body.



A ____________________

B ____________________

C ____________________

D ____________________

E ____________________

F ____________________

G ____________________

H ____________________

Figure 2-3. The nephron unit and collecting tubule (objective 605, exercise 11).

2-2. Physiology of the Kidney

Most laboratory technicians in the Air Force analyze many urine specimens every day. Yet, would most of us be able to explain how the body produces urine? Even more important in laboratory work, can we relate how physical properties of urine, for example, color and specific gravity have any real practical relationship to kidney function? If we are to describe urine and list its properties, it is only reasonable that we know why we are doing all of these tests. This section describes functions of the kidney and relates them to urine production.

606. Name the four functions of the kidney and describe these functions by citing structures and processes involved, controlling factors, and substances excreted and reabsorbed.

Formation of Urine. As a result of normal cellular metabolism, a number of nitrogenous wastes are formed. These include urea, uric acid, creatinine, and ammonia. Removing these wastes from the blood is one function of the kidney. It also regulates blood volume, controls the water content of tissue, and maintains the pH and chemical composition of body fluids. The process of urine formation begins with filtration of plasma-like fluid through the glomerular capillaries. The filtrate then passes down the tubules. It is reduced in volume and composition by tubular reabsorption and secretion which we will discuss in somewhat greater detail.

An important function of the kidney is glomerular filtration. Blood pressure of approximately 70 mm of mercury within the capillaries of Bowman's capsule is about twice that of any other capillary in the body. Opposing this force is an osmotic pressure of nearly 25 mm of mercury, which is normal capillary pressure. About 10 percent of the blood which passes through the glomerulus is removed in the form of glomerular filtrate. Approximately 1200 ml of whole blood pass through the kidneys each minute; thus, every 4 or 5 minutes a volume exceeding that of the total volume of blood in the body passes through the kidneys. Capillaries of the glomerulus are concerned only with filtration, not with reabsorption as are other capillaries of the body. Various mechanisms affect the rate of glomerular filtration. In the absence of compensating factors, a rise in blood pressure in the efferent arteriole increases the rate of filtration. This may be due to vasoconstriction of the efferent arteriole and possibly to an increase in systemic blood pressure. Glomerular blood flow is also under neutral control. For example, stimulation of the splanchnic (visceral) nerve decreases renal blood flow and, hence, reduces the rate of filtration. Vasodilators such as adrenalin also decrease glomerular filtration after an initial phase of vasoconstriction.

In the actual process of filtration, walls of the glomerular capillaries behave as filters. A very thin membrane, 0.1 μ thick, separates the blood from the cavity of the capsule. Substances having a molecular weight of 68,000 or less are able to pass through the membrane and are excreted. Proteins are normally retained. Thus, you can see why albuminuria (albumin in the urine) is generally considered abnormal. If small amounts of protein do pass through the glomerulus, the protein is reabsorbed in the proximal convoluted tubules. The pH of glomerular filtrate is about 7.4, with a normal specific gravity of 1.008 to 1.012 (average 1.010).

Reabsorption Phase. Most reabsorption takes place in the renal tubules. Many substances which are essential to life, including water, escape through the glomerular tuft. These substances must be replaced in the blood. In order to return them to the bloodstream, the convoluted tubules actively reabsorb some of these substances into the capillary network around the tubules. The proximal tubules reabsorb all of the glucose and 85 percent of the water from glomerular filtrate and about the same amount of sodium, bicarbonate, and chloride. If the plasma concentration of a solute exceeds a certain level, known as the tubular maximum, this substance appears in the urine. Other substances which are absorbed include amino acids and phosphates. Additional water reabsorption is thought to occur in the loop of Henle. After this, approximately 20 to 40 percent of the water remaining in the glomerular filtrate is reabsorbed in the distal tubules. Reabsorption in the distal tubules is under hormonal control, namely the antidiuretic hormone (ADH) of the pituitary gland.

In addition to reabsorption, the tubules excrete many substances including creatinine, ammonia, potassium, and foreign substances like penicillin and dyes. Most potassium found in the urine is excreted by the tubules, with the remainder having escaped in the glomerular filtrate without reabsorption. Incidentally, potassium is thought to be the only electrolyte which is actively excreted by the tubules. As the glomerular filtrate goes through these processes of filtration, reabsorption, and secretion, the total volume drops from the original 100 to 150 liters or more per 24 hours to about 1 to 1.5 liters of urine per 24 hours. The pH of urine is 4.6 to 8, while the average specific gravity increases from the original 1.010 to about 1.015 upward to 1.025.

Our discussion of kidney physiology would not be adequate without mentioning the importance of the kidneys in maintaining acid-base balance. Regulation of acid-base balance is accomplished by four mechanisms, not necessarily in order of importance. First, ketone bodies (acetoacetic acid, B-hydroxybutyric acid, and acetone) which may be present are oxidized to corresponding organic acids. These acids are subsequently excreted after conjugation with glycine as hippuric acid or phenylaceturic acid. By this means ketone bodies which cause acidosis are removed from the blood and excreted. Second, ammonium ions replace the more basic sodium ions to some extent. Ammonia from plasma amino acids and glutamine are released by enzymatic activity in the tubular cells of the kidney. Thus, losses are conserved by retention of sodium and substitute excretion of ammonium ions. Third, reabsorption of bicarbonate takes place in the tubules as previously indicated. Fourth, hydrogen ions are excreted by the tubules. The overall result is a change in the pH of glomerular filtrate from 7.4 to a range of 4.6 to 8.0 while the acid base balance of the body is maintained. The latitude of urine pH from acid to alkaline is to be expected and results from compensating mechanisms which control blood pH within a very narrow range.

Exercises (606):

1. Name four functions of the kidney.

2. Where does the process of urine formation begin?

3. What condition within Bowman's capsule makes possible glomerular filtration?

4. How does a change in blood pressure affect the rate of urine production?

5. Name two other factors that may affect the rate of filtration.

6. Why is albumin in the urine regarded as abnormal?

7. Where are most of the substances in the glomerular filtrate reabsorbed?

8. What happens when the plasma concentration of a solute exceeds a certain level known as the tubular maximum?

9. What controls reabsorption in the distal tubules?

10. Name five substances besides water that are reabsorbed.

11. Name four substances that are excreted.

12. What is the ratio of glomerular filtrate to urine excreted?

13. Name four mechanisms by which the kidneys maintain the acid-base or electrolyte balance of the body.

14. Compare the normal pH and specific gravity of glomerular filtrate with normal pH and specific gravity of urine.

2-3. The Kidney and Disease

Our attention will now be focused on some of the pathological conditions which either cause or are related to malfunction of the kidneys. As a student, your objective in studying this section is to realize what an abnormal laboratory report can mean to the physician, and thereby encourage you to render valid and intelligent reports. As repeated often in this course, clinical conclusions relative to a particular patient are beyond the scope of the laboratory.

607. Identify the conditions and terms related to diseases of the kidney.

Related Metabolic Diseases. You have on occasion seen the term *uremia* in the clinical block of laboratory report forms and elsewhere. It is a rather nonspecific term indicating the presence of abnormal quantities of urinary constituents in the blood. Uremia may result from practically any disease involving a malfunction of the kidneys and is represented by complex clinical symptoms. When uremia is diagnosed, provisionally or definitively, the laboratory is used to reinforce the diagnosis. In most cases there is an elevation of urea nitrogen in the blood and oliguria. However, not all of these signs and symptoms are always present. For example, the quantity of urine may be normal, in which case the serum sodium is usually increased. In the final analysis, laboratory data are meaningless unless one laboratory result is related to another. There is sometimes a question concerning the relative merits of particular procedures. If a choice exists between measuring urea nitrogen and nonprotein nitrogen, it would be advisable to select the urea nitrogen test because of its greater specificity. Nonprotein nitrogen measures creatinine, amino acids, and a composite of nitrogen-containing substances, as you may recall from your study of clinical chemistry.

There are many diseases of the glomeruli. There are also many recognized classifications of glomerular diseases. The type of glomerular disease is diagnosed partly on histological and partly on other laboratory evidence. For example, some glomerular diseases are accompanied by hematuria (blood in the urine) or proteinuria while others are not. This again emphasizes the need for careful and accurate laboratory work. You might also recognize that medical terms like glomerulonephritis have a limited and specific meaning. The term is not used to describe just any disease of the glomerulus. This is mentioned as a word of caution to urge you to form a meaningful medical vocabulary. You undoubtedly recognize that diseases not generally considered renal in nature may result in renal complications. For instance, nearly half of all patients with systemic lupus erythematosis (L.E.) have renal complications.

Diseases of the Tubules. A variety of kidney disorders are classified as diseases of the tubules.

Included in this category are mercury poisoning, gout, and other conditions, especially effects relating to toxicity. Also included among tubular disorders is multiple myeloma. Multiple myeloma is a malignant, tumorous condition of the bone marrow manifested in 40 to 50 percent of the cases by Bence Jones protein in the urine. However, myeloma is more than a disease of the bone marrow. Involvement extends to the spinal cord with consequent pyelonephritis. In addition, renal lesions develop although there is less certainty on the causal relationships between renal lesions and myeloma. Renal amyloidosis (starch-like deposits) is another aspect of this disease. The molecular weight of Bence Jones protein is in the range of 25,000 to 90,000. It, therefore, passes readily through the glomerulus if present and can be easily detected in the laboratory.

There are a variety of basic renal problems not previously discussed. In recent years there has been a resurgent interest in the effect of infections upon functions of the kidney. Infection may produce generalized and localized kidney damage, including interstitial nephritis and pyelonephritis. Typhoid, yellow fever, and tuberculosis are just a few diseases of special interest to a urologist. Another quite different problem which was developed in your study of clinical chemistry is the occurrence of renal calculi. Renal calculi are not as uncommon as you might think. They have been reported to occur in 5.4 percent of all autopsies, which is a good indication of their occurrence in the population. Next let us mention tumors and malignant neoplasms, such as lymphatic leukemia, to illustrate that other conditions have a profound influence on kidney pathology. Unless you are a histopathology technician, you will probably not directly observe the effects of these conditions on tissue. However, the effects will be apparent from other tests which you will perform.

Finally, in this category of miscellaneous diseases which affect the kidneys can be included diseases of the blood vessels and diabetes, a disease which you studied in Chapter 4, Volume 2, of this course. There are at least a dozen well-known vascular diseases which include glomerulosclerosis, nephrosclerosis, arteriosclerosis, and thrombosis. These usually involve the capillaries, arterioles, arteries and veins, respectively. In diabetes mellitus a high blood sugar results from insufficient production of insulin by the pancreas. When the glucose level of the blood exceeds that which can be reabsorbed by the tubules, the renal threshold has been exceeded and glucose appears in the urine. Kidney damage results from accompanying effects of the disease. Keep in mind that glucose in the urine is only one of various symptoms.

Exercises (607):

Match the disease terms in column B with the conditions to which they closely relate in column A by placing the letter of the column B item beside the number of the column A item. Each column B item may be used once or more than once.

Column A

____ 1. Vascular disease which primarily involves the capillaries.

____ 2. Vascular disease which primarily involves the arterioles.

____ 3. Indicates the presence of urinary constituents in the blood.

____ 4. An aspect of multiple myeloma resulting in the formation of starch-like deposits in the tubules.

____ 5. Blood in the urine.

____ 6. Considered a tubular disorder, in which up to 50 percent of the cases show Bence Jones protein in the urine.

____ 7. Infections producing localized or generalized kidney damage.

____ 8. Malignant neoplasm having profound influence on kidney pathology.

Column B

a. Uremia.
b. Hematuria.
c. Multiple myeloma.
d. Renal amyloidosis.
e. Lymphatic leukemia.
f. Pyelonephritis.
g. Interstitial nephritis.
h. Glomerulosclerosis.
i. Nephrosclerosis.

CHAPTER 3

Physical Characteristics of Urine

A REPORT CONCERNING the physical characteristics of urine is a part of every complete urinalysis. The macroscopic examination includes total volume, color, appearance, pH, and specific gravity. In this chapter we will discuss all of these tests and their physiological significance. We shall also describe techniques generally used in the laboratory to evaluate the physical characteristics of urine.

3-1. Urine Volume, Color, and Appearance

The rate of urine formation and, accordingly, the volume is subject to many wide fluctuations. Many factors operative in healthy persons, as well as in persons with pathological conditions, may cause variation and occasionally provide a warning of abnormality.

608. Cite normal and abnormal volumes of urine output, identify physiological factors and pathological conditions associated with abnormal volumes, and tell the significance of volume in relation to given laboratory determinations.

Volume. The total urine volume voided in 24 hours varies with diet, body size, fluid intake, fluid loss in perspiration from temperature or exercise, and the ingestion of diuretics, such as coffee. The average total daily volume is about 1,200 to 1,500 ml from 10 years of age through adulthood. However, in children the total volume in relation to body size is increased considerably. The maximum total daily output of urine should not normally exceed 2,000 ml in adults.

Polyuria. If a volume higher than 2,000 ml in adults is excreted, the condition is called polyuria. Polyuria may be a physiologic response to increased fluid intake; the ingestion of diuretic medications or certain diuretic drinks, such as coffee, tea, and alcohol; chilling of the body; nervousness and anxiety; or the intravenous infusion of fluids. Polyuria also occurs in several disease conditions, particularly in diabetes mellitus and diabetes insipidus. It is a symptom of chronic renal disease and has been noted in patients with certain tumors of the brain and spinal cord, acromegaly, and myxedema. Polyuria may indicate the loss of concentrating ability of the kidneys.

Oliguria. Oliguria is a decreased urinary output of less than 200 ml/24 hours. The extreme form in which there is a total lack of urine is called anuria. Physiologic forms of oliguria occur with decreased fluid intake, increased ingestion of salt, and excessive sweating. Oliguria occurs when there is excessive loss of body fluid as in vomiting, fever, bleeding hemorrhoids, and diarrhea, or when there is renal shutdown either through inflammation (nephritis), poisoning, or in cardiac insufficiency. Occasionally oliguria occurs due to mechanical obstruction to the urinary flow. There is normally a decreased total urinary output during the night, probably due to decreased intake of fluid.

Importance of volume in urinalysis. Random specimens are most often requested for routine urinalysis. The volume varies considerably, but this is unimportant as long as the minimum amount (usually 15 ml) is obtained. A fresh, concentrated, first-morning specimen is undoubtedly the most valuable specimen to the physician. This is particularly true in regard to the formed elements, that is, cellular components. The specimen should never be discarded as QNS (quantity not sufficient) without performing as many of the requested tests as possible. This usually includes all of the routine examinations, except perhaps specific gravity. However, even the specific gravity can be determined as long as the volume is sufficient to dilute. The quantity of urine obtained for analysis is a particular problem with children and infants. As a technician, you have a responsibility to assist parents in obtaining an adequate specimen. However, other laboratory tests (for example, blood count) should not be delayed because you must wait to obtain a urine specimen.

Exercises (608):

Match each statement about urine volume in column A with the term or response most closely related to it in column B by writing the letter of the column B item in the appropriate space in column A.

	Column A	*Column B*
___	1. Average daily volume of urine excreted by an adult.	a. Blood count. b. Anuria. c. Increased fluid intake,

Column A

____ 2. Condition in which there is increased output of urine.
____ 3. Condition in which there is decreased output of urine.
____ 4. Condition in which there is a total lack of urine output.
____ 5. Physiological factors that may cause polyguria.
____ 6. Pathological conditions associated with polyguria.
____ 7. Physiological factors that may cause oliguria.
____ 8. Pathological conditions associated with oliguria.
____ 9. Type of specimen most often requested for routine urinalysis.
____ 10. Type of specimen most valuable to physician, particularly with regard to formed elements.
____ 11. Determination which can be made regardless of volume by dilution of specimen.
____ 12. Determination that should not be delayed because volume of specimen is insufficient.

Column B

diuretic medication or drinks, nervousness, or intravenous infusion of fluids.
d. Specific gravity.
e. Oliguria.
f. Random specimen.
g. Diabetes mellitus or diabetes insipidus.
h. Decreased fluid intake, increased ingestion of salt, and sweating.
i. 1,200 to 1,500 ml.
j. Vomiting, fever, diarrhea, nephritis, cardiac insufficiency.
k. First morning specimen.
l. Polyuria.
m. 1900-2500 ml.

609. Identify the conditions that produce urine color and appearance and show their relationship to disease conditions.

Color. Urine color varies considerably in healthy and diseased states. Normally, urine is some shade of yellow. This yellow may be almost colorless, yellow-green or straw colored, pale yellow, bright yellow, light amber, or dark amber. Variations in body metabolism and certain vegetable pigments from the diet will alter these colors. Chromogens in beets, for instance, may produce red urine; while carrots give a bright yellow color (carotene) to the specimen. Certain drugs or their metabolites may also produce colored urine; for example, methylene blue dye and various sulfa drugs. These pigments may be so concentrated that they confuse color comparisons made in urine strip screening tests; for example, Labstix®, Hema-Combistix®, Multistix®, Tes-tape®. In this case several alternatives are possible. Chromogens may be removed by mixing with activated charcoal and filtering. This filtrate can then be screened for glucose. However, since normal chromogens are also removed by activated charcoal, the pH is probably altered in this process and protein analysis may be invalidated. Qualitative protein analysis may be determined with heat and acetic acid on the untreated specimen. It may also be stated that light-colored specimens are usually lower in specific gravity than darker specimens. There are exceptions, though, as in the case of diabetes, where the urine may be straw colored or pale yellow in color and yet have a high specific gravity due to the presence of glucose.

Pathological urine specimens, then, may have the same color as normal urine. A green-yellow specimen may result either from normal metabolism or from the presence of bile or bacterial chromogens, for example, Pseudomonas infection. Red urine is produced from medications like PSP (phenolsulfonphthalein) dye or pyridium, which are often used in kidney disease diagnosis or therapy. Red color may also be caused by the presence of intact red blood cells (hematuria) or hemoglobin derived from red cells (hemoglobinuria). Porphyrins impart a wine red color to urine (porphyrinuria). Remember, though, a red color can appear after the ingestion of beets. Alkaptonuria is characterized by the presence of alkapton bodies (homogentisic acid) and a consequent black urine when the urine specimen is made alkaline or becomes alkaline upon standing. Alkaptonuria occurs because of an inborn error in the metabolism of two amino acids, phenylalanine and tyrosine. Black urine is seen also when melanin, the pigment of hair and skin, is present as the result of certain malignancies. Finally, pus, certain crystals, chyle (fat), and some bacteria give a milky appearance to urine. This condition will be discussed at greater length in the following paragraph. Definite assumptions as to the presence of pathology cannot be made solely on the basis of the color of urine. However, abnormal urine color should alert you to the possibility of pathology which may be confirmed or denied by more specific tests and the physician's findings in examination of the patient.

Appearance. The appearance of urine refers to another physical characteristic which is routinely observed and reported. The term "appearance" refers to the transparency of the specimen. "Clear," "hazy," and "cloudy" are descriptive terms used for this report. Suspended crystalline particles of phosphates, urates, and carbonates are often the cause of haze or cloudiness. However, heat may be used to dissolve urates, and acetic acid may be used to dissolve carbonates and phosphates. Cellular material, including epithelial cells, blood, pus, and bacteria, will also cloud the urine specimen. These substances, as well as insoluble crystalline salts, can be removed by centrifugation. Confirmation of the nature of the material causing cloudiness is usually done microscopically. Rarely, chyluria (fat in the urine) may cause a specimen to appear cloudy, and this is also confirmed microscopically. Chyle is easily removed by (1) adding ether to dissolve the fat and (2) separating the aqueous/ether phases in a separatory funnel.

Exercises (609):

1. List three conditions that may change the normal color of urine.

2. Why do these abnormal colors present a problem in the laboratory?

In exercises 3 through 9, match the abnormal color or appearance of the urine in column A with the conditions that may produce them. Each column A item may have one or more than one correct response.

	Column A	*Column B*
____	3. Red color.	a. Presence of bile or Pseudomonas infection.
____	4. Black color.	b. Ingestion of beets.
____	5. Green-yellow color.	c. Alkapton or melanin.
____	6. Wine-red color.	d. Hematuria or hemoglobinuria.
____	7. Milky or cloudy appearance.	e. Prophyrinuria.
____	8. Pale yellow or straw color.	f. Chyle (fat).
____	9. Bright yellow color.	g. Ingestion of carrots.
		h. Cellular material such as epithelial cells, pus, and bacteria.
		i. Diabetes.

10. How may chromogen be removed from a urine specimen?

11. What assumption of pathology can you make solely on the basis of urine color?

In exercises 12 through 15, match the substance that causes a urine specimen to be cloudy in column A with the appropriate procedure that will remove the substance in column B.

	Column A	*Column B*
____	12. Urates.	a. Centrifugation.
____	13. Carbonates and phosphates.	b. Heat.
____	14. Cellular material and insoluble crystalline salts.	c. Adding ether and separating the aqueous/ether phases in a separatory funnel.
____	15. Chyle.	d. Acetic acid.

3-2. Reaction and Specific Gravity

The kidney regulates the selective excretion of the various cations in order to maintain normal acid-base balance. In addition, a balance is also maintained by the specific gravity which indicates the relative proportions of dissolved solid components to the total volume of the specimen. This section reviews common techniques used to evaluate the pH reaction and specific gravity of urine.

610. Indicate the changes in pH reaction of urine specimens when allowed to stand at room temperature as the result of disease conditions and as treatment ordered by the physician in given conditions.

Reaction. The reaction (pH) of freshly voided normal urine varies from 4.8 to 8.0 with a mean of approximately 6.0. When the specimen stands at room temperature, urine becomes less acid (pH becomes numerically higher) by the formation of ammonia from urea, which is a normal chemical constituent of urine. The urea is chemically split by bacteria to form ammonia. The concentration of ammonia in old urine specimens can become so high as to be noticeable from its distinctive odor. A marked pH shift to alkaline in aged urine interferes with certain qualitative screening tests—for example, protein stick tests—and destroys important microscopic cellular components. This is the principal reason for performing a routine urinalysis only on a fresh urine specimen. The pH is of clinical diagnostic significance only in a freshly voided or catheterized specimen.

Strongly acid urine (pH lower than 6.5) is encountered in cases of metabolic acidosis—the ketosis of diabetes mellitus, for example. In addition, strongly acid urine reactions are found in gout, acute rheumatism, chronic nephritis, tuberculosis of the kidney, fever, leukemia, and inflammations of the heart, liver, kidneys, and lungs. Dehydration causes an acid reaction because of the loss in liquid volume without a simultaneous reduction in acid (hydrogen ion) excretion by the kidneys. Finally, an acid reaction in urine may occur from the ingestion of a high-protein diet.

Urinary tract infection is the most common cause of persistent strongly alkaline urine. Again, this occurs because of the ability of urinary bacterial pathogens to split urea to form ammonia. The microorganism *Proteus vulgaris* is most frequently identified in this connection. An alkaline reaction is also found with persistent vomiting (gastric acidity is reduced), in certain anemias, some cases of debility, cystitis, and in cases of obstructive ulcers.

As noted in the preceding paragraphs, urinary pH may be altered by several pathological processes. The physician may also intentionally alter the urine pH in conjunction with therapy. This is generally the situation in patients suffering from the formation of urinary calculi. You learned in Volume 3 of this course about the occurrence of urinary calculi, and that certain calculi, specifically oxalate, uric acid, and cystine stones, form in an acid urine. In treatment, then, the physician adjusts the urine pH through proper diet and drugs to maintain a persistently alkaline urine. This eliminates the chemical environment in which these acid stones form. Conversely, phosphate and carbonate stones, formed in alkaline urine, are treated by creating in the urinary tract an acid urine medium which inhibits their formation. Another instance in which the physician controls urine pH is in the treatment of a urinary tract infection with the antibiotic mandelamine. Mandelamine is effectively bactericidal only when an acid pH is maintained. In therapy with the sulfonamides, an alkaline urine is created to prevent precipitation of sulfa crystals and consequent damage to the urinary tract.

As you see, urinary pH can be a helpful indicator for some physiological functions, and it adds one more parameter to the physician's clinical picture of a patient. Also, we noted that in some instances urinary pH measurements are essential for adequate treatment of the patient. Of course, your responsibility to the clinician and patient is to know the technical aspects of urinary pH measurement and to be able to determine pH accurately and quickly.

Exercises (610):

1. What change occurs in the normal reaction of fresh urine upon standing at room temperature?

2. What kind of reaction is indicated in conditions of gout, acute rheumatism, chronic nephritis, tuberculosis of the kidney, fever, and leukemia?

3. What kind of reaction is noted in a urinary infection, or persistent vomiting?

4. Why might a physician intentionally alter the pH range of a patient?

611. Show the principle used in measuring urine pH, and identify possible sources of errors.

Urine pH Measurement. For routine analysis, urinary pH may be measured with indicator paper strips and a color chart. When more exact determinations are needed, a pH meter is used and the answer is obtained directly from the meter.

A variety of test papers impregnated with various chemicals are available for the easy and rapid colorimetric determination of pH. Since urine pH is almost always measured as part of the more complete urinalysis, it is advantageous to use a multiple determination reagent strip, such as COMBISTIX® Reagent Strip, LABSTIX® Reagent Strip, BILI-LABSTIX® Reagent Strip, or MULTISTIX® Reagent Strip, that simultaneously measures pH and checks the urine for several other components.

The pH portion of each of these strips is impregnated with two separate indicators, methyl red and bromthymol blue. These chemicals provide a wide spectrum of color changes, from orange to green to blue, in the pH range of 5 to 9. The reagent strip is dipped into the urine specimen, and the color change is compared to the standardized color charts on the bottle labels which show pH values 5 through 9 in steps of one pH unit. Any color obtained may be interpolated to one-half pH unit.

Nitrazine indicator papers are sensitive and specific in the pH range of 4.0 to 8.0. Several wide-range pH papers are also available but are not particularly useful for determinations of urinary pH because the increments on the color scale are too great for close pH approximations.

Nitrazine indicator papers are sensitive and specific in the pH range of 4.0 to8.0. Several wide-range pH papers are also available but are not particularly useful for determinations of urinary pH because the increments on the color scale are too great for close pH approximations.

Sources of Error. Several sources of error must be kept in mind when you use these strips. First, the strips should be kept in the sealed dispenser and away from strong acid or alkaline fumes when not in use. Secondly, the strips should be kept dry until they are used. Then when you actually measure pH, you should not soak the indicator strip in the solution you are testing. Obviously, this means that you should not drop an indicator strip into the urine. If the strip is soaked in urine, the indicator reagent will be leached from the paper and the pH will be impossible to read. The correct way is to dip one end of the strip into the urine. Dip it only long enough to saturate the end portion, and drain it immediately by touching the strip to the side of the container. The strip should not be placed on the lab bench after wetting because of a good possibility of contaminating the reaction with residual chemicals on the bench.

Sanitation of the workbench is another consideration. These reagent strips are designed exclusively for qualitative and semiquantitative measurement of specific constituents in urine. Each reagent strip consists of a strip of cellulose with a series of small squares of reagent-impregnated paper attached. The cellulose is nonwettable and therefore easily drained, so that the reagent squares are not soaked by an excess of specimen. In addition to the sources of error mentioned in the previous paragraph, it should be noted that these strips are packaged in a screw-capped bottle which contains a desiccant (drying) chemical. The bottle must be kept tightly closed if the reagents are to remain dry and stable. Also, the desiccant packet must be left in the storage bottle. If these precautions are not observed, atmospheric moisture will be absorbed by the strips, and the reagents will deteriorate.

Exercises (611):

1. With what two indicators is the reagent strip impregnated and what is the pH range?

2. Even though specific and sensitive, why are nitrazine indicator papers not useful for urinary pH measurement?

3. List three sources of error which may be encountered using pH paper strips.

4. Why should the desiccant pack be left in a urine reagent test strip bottle?

612. Indicate whether given statements correctly reflect the definition of specific gravity and the techniques for calibrating and using the urinometer.

Specific Gravity. "The ratio of the weight or mass of a given volume of a substance to the weight of an equal volume of a standard; that is, pure water," defines specific gravity. For practical purposes, 1 cc (cm^3, cubic centimeter) of pure water weighs 1 gram (1.000 g) at 4°C to three decimal places. If this cubic centimeter of urine weighs 1.010 g, then we can assume by the definition that the sp gr is a ratio of $\frac{1.010}{1.000}$ and the sp gr is 1.010. Actually, this procedure is impractical for routine use with multiple specimens. However, another way to determine specific gravity is to measure the displacement of a fluid by a solid of constant weight immersed in the fluid. This is the principle of the urinometer (hydrometer) pictured in figure 3-1.

The urinometer is calibrated in distilled water at the temperature specified on the stem by the manufacturer. The sp gr of distilled water at the calibration temperature should be 1.000. For each 3°C of specimen temperature above the calibration temperature, 0.001 is added to the sp gr reading obtained. Of course, this value is subtracted for each 3°C less than calibration temperature. All new urinometers should be checked for accuracy, and mathematical corrections should be made for slight inaccuracies. If the calibration value is grossly inaccurate, the urinometer should be discarded.

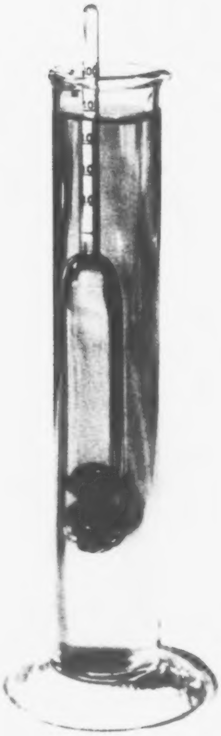


Figure 3-1. Urinometer (hydrometer).

The technique of using a urinometer is quite simple. First, bring the urine specimens and water for calibration to room temperature. If this is not done, the temperature of each specimen should be determined at the time the sp gr is read and suitable correction made. After this, a volume of specimen sufficient to float the urinometer is placed in the glass cylinder. The urinometer must not touch the bottom of the cylinder. Then spin the urinometer in the urine and take a reading at the bottom of the meniscus before the urinometer stops turning and settles to the side of the cylinder. Read the result to the nearest 0.001 unit. Then add or subtract the calibration value and record the specific gravity.

The minimum volume of urine necessary to float a urinometer is approximately 15 ml. This varies somewhat, depending upon the sp gr and the size of the urinometer. You should calculate the dilution to be made by measuring the volume of urine available. For instance, if 10 ml of specimen are obtained, a twofold dilution with distilled water results in a final volume of 20 ml (10 ml urine + 10 ml H_2O). This is more than 15 ml and sufficient to float the urinometer. For a twofold dilution the three digits following the decimal point must be doubled when the sp gr is read. For

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instance, if the reading on the urinometer is 1.004 after correction for temperature, the actual sp gr would be 1.004 + 0.004, or 1.008. If the specimen is diluted threefold (that is, 5 ml urine + 10 ml distilled water), then the last three digits would be tripled. Thus, a temperature corrected reading of 1.003 on a threefold dilution would mean a sp gr of 1.009 for the undiluted urine. The glass cylinder may be rinsed with tap water between specimens and *must* be rinsed after very cloudy specimens to facilitate later readings.

Exercises (612):

Indicate whether each sentence is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. Specific gravity is the ratio of the weight or mass of a given volume of a substance to the weight of an equal volume of a standard such as pure water.

T F 2. 0.003 is added to or subtracted from the observed reading of each 3°C higher or lower than the calibration temperature.

T F 3. For a threefold dilution of a specimen, the three digits after the decimal point would be multiplied by 4.

T F 4. A reading of 1.006 with a twofold dilution would be 1.024.

T F 5. It is not necessary to check all urinometers for accuracy and mathematical corrections.

613. Indicate the principle of the refractive index by citing the definition, correlation with specific gravity, clinical use, accuracy of the urinometer, and three advantages of the refractometer.

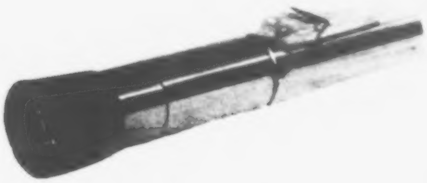


Figure 3-2. Clinical model refractometer.

Refractometric Measurement of Specific Gravity. There is a direct relationship between the concentration of dissolved solids and the refractive index of a solution. By definition, specific gravity is a ratio of the weight (or mass) per unit volume, so specific gravity is also related to the refractive index of a solution. A refractometer is an instrument designed to measure refractive index.

The refractive index of urine correlates closely with the specific gravity. Consequently, in some instances, measurement of the refractive index may be substituted for measurement of specific gravity. This is especially useful for small urine specimens, since measurements can be made on as little as one drop of urine. The refractive index is the ratio of the velocity of light in air to the velocity of light in solution. This ratio varies directly with the number of dissolved particles in urine and, as such, varies similarly with the specific gravity of urine. The most useful clinical application of the principle of the refractometer is the determination of specific gravity in small quantities of urine.

A small clinical refractometer developed in recent years has replaced the urinometer in many laboratories. This instrument is calibrated in terms of specific gravity, refractive index, and total solid content. It utilizes only a drop of urine placed in the appropriate spot in the refractometer. The instrument is held towards a light source and the answer, whether in terms of specific gravity, refractive index, or solid content, is read directly from the calibrated scale located in the eyepiece.

Figure 3-2 is a photograph of the clinical model refractometer. This instrument will accurately measure urine specific gravity to the nearest 0.001 unit. It has an advantage in that it requires only 0.02 ml of specimen and is easily loaded with urine. Because of the small sample size, the specimen assumes ambient (room) temperature immediately, and the corrected value can be read directly from an internal scale. Calibration is easily checked against the refractive index of water. The efficiency in time saved in taking multiple specific gravity readings is a great asset with this instrument. Of course, freedom from tedious manipulations is also advantageous. Special care should be exercised in using this delicate optical instrument. Holders are available to help prevent accidentally dropping the refractometer while it is in use. If a holder is not used, the instrument should be stored in the case provided by the manufacturer. In no circumstance should the refractometer be left on the lab workbench where it might be damaged or pilfered.

Exercises (613):

1. Define refractive index.

2. There is a direct relationship between the refractive index of a solution and the concentration of _________ .

3. For what purpose is the refractive index most clinically useful?

4. How accurately does the urinometer measure specific gravity?

5. List three of the four advantages of the use of the refractometer.

3-3. Osmolality of Urine

Volume 3, chapter 1, discussed methods of measuring urine concentration, including the test of osmolality. This section provides you with more specific information concerning urine osmolality. The osmolality of urine is a more exact measurement of urine concentration than is specific gravity.

614. Indicate whether given statements correctly reflect the measurement of urine osmolality.

Measurement of Osmolality. The osmolality of urine is an indicator of the amount of osmotic work done by the kidneys. Osmolality depends on the number of particles of solute in a unit of a solution, whereas specific gravity depends on both the quantity and the precise nature of the particles in solution. Large, dense particles such as protein, sugar, and intravenous dyes elevate urine specific gravity disproportionately more than the osmolality. Because the determination of specific gravity is so simple and easy to perform, this measurement is generally performed in the routine laboratory analysis and serves quite adequately for the majority of specimens. Whenever a more precise measurement is indicated, osmolality of the urine can be determined.

The osmolality of a solution is a measure of the number of osmols in one kilogram of solution. Thus, it is a measure of the number of particles in a given weight. This is often confused with osmolar solutions; osmolarity is a measure of the number of particles in a given volume of solution—osmols per liter of solution. The osmolality and osmolarity of relatively dilute solutions, such as urine, are practically identical, and the differences are generally neglected in routine laboratory examinations.

Plasma/urine osmolality, osmolal clearance, and free water clearance. Evaluation of the concentrating and diluting ability of the kidney can progress beyond routine clinical measurements to determinations of the ratio of plasma to urine osmolality, the osmolal clearance, and the free water. The ratio of plasma/urine osmolality measures the concentrating ability of the kidney and normally ranges from 3.0 to 4.7. The osmolal clearance reflects the ability of the kidney to conserve or excrete water. It equals the ratio of the urine osmolality to the product of the plasma osmolality multiplied by the rate of urine flow in ml/min. The free water clearance is a better expression of this kidney function. Free water clearance equals the urine flow, in ml/min, minus the osmolal clearance.

When "free" water is excreted, urine osmolality is less than plasma osmolality; when water is being retained, urine osmolality is greater than plasma osmolality. Free water clearance is negative during tests of concentrating ability and is decreased when less plasma filtrate passes through the glomerular membrane, when there is excessive secretion of antidiuretic hormone, and in patients with heart failure and liver damage. Free water clearance is increased in tests of diluting ability and is elevated in patients with diabetes insipidus, adrenal insufficiency, and certain head injuries.

Average normal values. Normal kidneys are capable of diluting and concentrating urine from a minimal range of 40 to 80 mOsm/kg water during a water diuresis to a concentration of 800 to a maximum of 1400 mOsm/kg water with fluid deprivation. The normal range of urine concentration for a patient on a normal fluid and food intake is from 500 to 850 mOsm/kg water.

Methods of Determination. Osmotic pressure is measured indirectly by determining depression of either the freezing point or the vapor pressure of the urine. The differences between the freezing points and vapor pressures of water and of an aqueous solution (in this instance, urine) are directly proportional to the molality of the solutions. A one molal solution, 1000 mOsm/kg, depresses the freezing point 1.86°C below the 0°C freezing point of water.

Osmolality can be determined on as little as 3 ml of urine by measuring freezing point depression with a freezing point osmometer (see fig. 3-3). These instruments are calibrated for both temperature and osmolality readings.

Until recently, freezing point depression was the only practical method of determining osmolality. A vapor pressure osmometer is now available also. It is relatively simple and easy to use and requires only a few drops of urine for the determination. An advantage of this method is that osmolality can now be measured at any selected temperature.

380

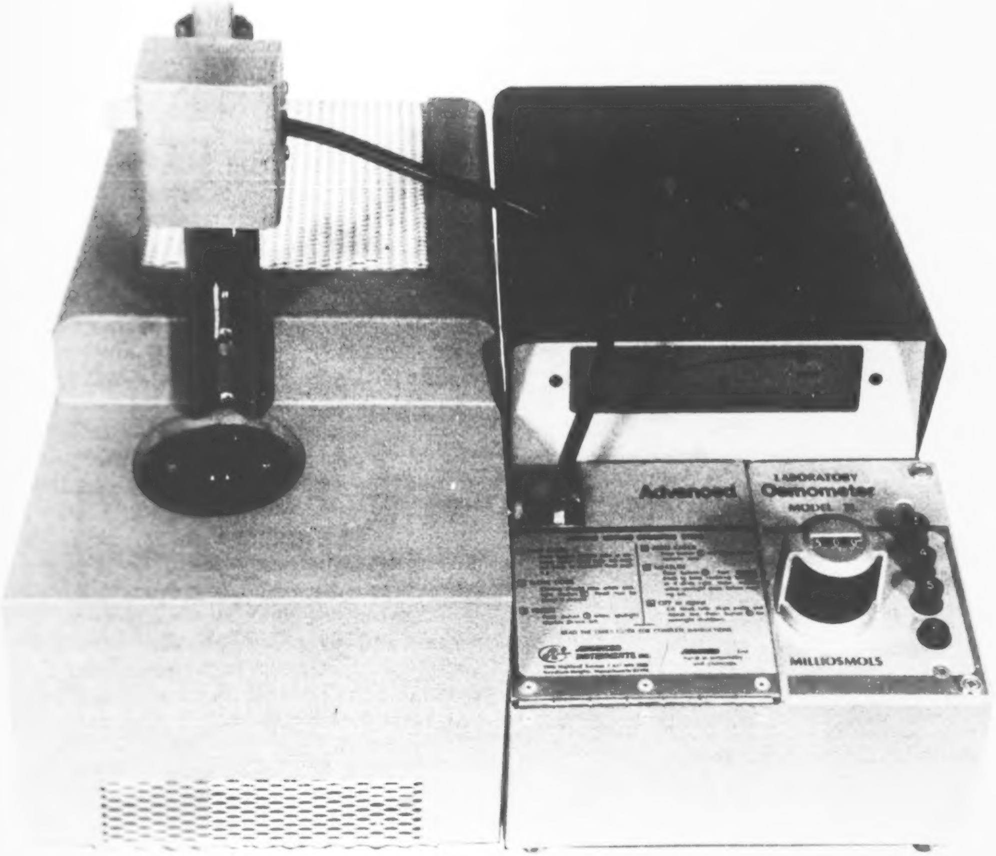


Figure 3-3. Freezing point depression osometer.

Exercises (614):

Indicate whether each sentence is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. The osmolality of urine is an indicator of the amount of osmotic work done by the bladder.

T F 2. Osmolality depends on the number of particles of solute in a unit of a solution.

T F 3. When compared to the specific gravity the osmolality is equally dependent on the quantity and the precise nature of particles in a solution.

T F 4. Measurement of osmolality of a solution is a measurement of the number of osmols in one gram of solution.

T F 5. Osmolality is based on weight of solvent and osmolarity is based on volume of solvent.

T F 6. The osmolal clearance reflects the ability of the kidney to concentrate fluids.

T F 7. Free water clearance equals the urine flow in ml/min, minus the osmolal clearance.

T F 8. Free water clearance is elevated in patients with diabetes insipidus, adrenal insufficiency, and certain head injuries.

T F 9. The normal range of urine concentration for a patient on a normal fluid and food intake is from 500 to 850 mOsm/kg water.

T F 10. Measurement of osmotic pressure is obtained indirectly by determining depression of either the freezing point or the vapor pressure of the urine.

T F 11. The differences between the freezing points and vapor pressures of water and of an aqueous solution (urine) are inversely proportional to the molality of the solutions.

T F 12. One advantage of the vapor pressure osmometer is that osmolality can be measured at any selected temperature.

CHAPTER 4

Microscopic Examination

IN CHAPTER 1 we learned the proper method for establishing critical microscopic illumination. We will now discuss some of the important components of urine which can be observed and identified with an adequate microscope system. The biological origin of these components and their relevance to health and disease relate to the structure and functions of the human excretory system described in Chapter 2. Our attention now turns from the gross physical features outlined in Chapter 3 to a systematic examination of urinary sediment as another valuable tool to aid the physician in diagnosing and treating the patient.

In addition to these important points which were discussed in the preceding chapters, it is recognized that technicians may not possess adequate knowledge of structures present in the sediment. In this chapter we will consider both cellular (organized) crystalline and amorphous urinary sediments. The use of stains in obtaining consistent and reliable microscopic results will be emphasized. Also included is the general significance of normal and abnormal findings.

4-1. Organized Sediments

Microscopic examination of fresh urinary sediment should be done routinely. A qualitative or semiquantitive evaluation of urine sediment generally provides adequate information for a majority of diagnostic and clinical needs.

615. Define the term "organized sediment," list common types of organized sediment, and tell how you can distinguish between organized sediment and other organic materials found in urinary sediments.

Definition and Types. Body cells and their derivatives are referred to as *organized sediment*. The common types of organized sediment include blood cells, epithelial cells, spermatozoa, and casts. Such structures may be found in small numbers in most urine specimens, but if present in large quantities, they usually indicate a pathological condition. Other organic materials that may be found in urine include mucus, bacteria, yeasts, parasites, and fat globules.

Various contaminants often resemble organized sediment. Talcum powder, starch granules, and oil droplets are sometimes mistaken for blood cells. Starch granules vary in size and shape and turn blue-black upon addition of iodine. Oil droplets are spherical and show concentric rings of light refraction upon focusing up and down with the fine adjustment. Pollen grains may be confused with erythrocytes or parasites. They vary in size and appearance according to their source. Generally such plant material can be distinguished from animal cells by the comparatively thick cell wall and lack of organized internal morphology. Yeast from urine bottles can confuse a microscopic examination, although the use of disposable paper containers reduces the potential problem. Yeast cells can usually be identified by their characteristic budding. Even air bubbles may be mistaken for cells. It is helpful to rotate the eyepiece periodically to be certain that extraneous structures adhering to the glass are not being identified as objects in the specimen.

Exercises (615):

1. What is meant by "organized urinary sediment"?

2. What are the common types of organized sediments?

3. How can you easily identify starch granules which might appear in urine?

4. How are the yeast cells distinguished from other elements such as red blood cells?

616. Point out the procedures for preparing specimens for microscopic analysis by citing the desirability of centrifugation, methods of clearing specimens, and desirability and methods of staining.

Preparing the Specimen. In most hospital laboratories, large numbers of specimens for microscopic examination arrive in the laboratory in groups and individually throughout the day. There is generally no reason to hold these specimens or preserve them. Perhaps the greatest problem is keeping them properly identified and separated from "stat" requests. In a well-organized laboratory, urinalysis will begin with the first group of specimens which can be conveniently processed. In other words, don't wait until 0900 hours to examine specimens that arrive at 0700 hours. Formed elements may disintegrate and valuable information will be lost.

Centrifugation. It is necessary to centrifuge all specimens for microscopic analysis? While some workers contend that it is *not* desirable to centrifuge urine specimens, experience has shown that centrifugation is probably the best approach. There is a chance that not all significant sediment will be observed on direct examination of urine. Centrifugation will not destroy fragile cells if the specimen is not centrifuged excessively. On the other hand, relatively rare elements such as casts, may be missed altogether if the specimen is not centrifuged. In most laboratories, it is considered best to centrifuge 10 ml of urine and report all elements except casts in number per high power field. It is left to local policy to decide which specimens, if any, can be observed directly without centrifugation. Coverslips should be used to enhance uniform distribution of the sediment which is transferred from the centrifuge tube to a corresponding slide. The entire specimen must be thoroughly mixed before you pour a portion of it into a centrifuge tube. There should never be more than two specimens on any one slide. The use of Boerner slides is not recommended because these slides do not permit the proper use of coverslips.

Clearing of specimen. Urine may be cleared of amorphous carbonates and phosphates by adding a few drops of dilute acetic acid (less than 10 percent) to 10 ml of urine before centrifuging. This will permit a clear view of significant structures. However, be careful not to add more acid than necessary to clear urine because casts and erythrocytes may also dissolve. The urine may be cleared of amorphous urates by mixing the urine with equal parts of warm (38°C) physiological saline before centrifuging. If less than 10 ml of the urine are centrifuged, you will need to make a correction for dilution. Of course, if you use 10 ml of urine in addition to diluent, no correction is necessary.

Staining urinary sediment. Before discussing the appearance of structures in the urine, we must decide whether we will be viewing stained or unstained sediment. The use of stain is recommended because it permits rapid and accurate identification of organized sediment. There are no real objections to using a stain except lack of training in this regard on the part of the technician. Best known of the supravital stains is the so-called Sternheimer-Malbin stain. The working stain consists of three parts solution A and 97 parts solution B, and is stable for several weeks if it is filtered as required. Stock solutions A and B are stable indefinitely when kept in separate containers, and consist of the following ingredients:

Solution A

Methylrosaniline chloride (crystal violet)	3.0 g
Ethyl alcohol, 95 percent	20.0 ml
Ammonium oxalate 0.8 g and distilled water to	80.0 ml

Solution B

Safranine 0	0.25 g
Ethyl alcohol 95 percent	10.0 ml
Distilled water to	100.0 ml

The stain is not difficult to use since it can be kept in a dropper bottle and a few drops can be added to the sediment in the centrifuge tube. Once you are familiar with observing stained sediment, it will be easy to observe and identify the various types of organized sediment. As emphasized in Chapter 1 of this volume, without critical illumination it is virtually impossible to accurately perform a urinary microscopic examination. Some of the difficulties associated with illumination can be overcome by staining the sediment. Throughout the remainder of this chapter, we will describe sediment in both the stained and unstained conditions.

Exercises (616):

1. Why is centrifugation a better approach for observing urine sediment than direct examination?

2. How many specimens should be placed on one slide?

3. How many milliliters of urine should be centrifuged and how are the elements, including casts, reported?

4. The addition of dilute acetic acid is recommended to clear urine of amorphous phosphates. Why is the amount critical?

5. How would you clear amorphous urates from urine?

6. What does the working stain solution of the Sternheimer-Malbin stain consist of?

7. What two main stains are comprised in the Sternheimer-Malbin stain?

617. Indicate types of epithelial cells and white blood cells found in urine specimens, their origins, terms used to describe them, their significance, and methods of differentiation.

Epithelial Cells. We will not attempt to distinguish among the types of epithelial cells on the basis of pathology alone. It is now believed that it is difficult, if not impossible, to state where in the urinary tract epithelial cells originate. You should be extremely cautious in attempting to define the origin of a particular cell. The term "renal epithelial cell" is often used to indicate a rounded type of cell. The term "renal" may be a misnomer in some cases because these cells do not always come from the kidney. Various types of epithelial cells are shown in detail A of foldout 1, which you will find at the back of the volume. However, you should not infer that cells which appear rounded necessarily had their origin in the kidneys. On the other hand, it is true that large numbers of these cells will be found in chronic kidney disorders, in nephritis, and in nephrosis. When epithelial cells are noted in casts, they are presumed to arise from the kidney tubules.

It is difficult to state the exact significance of epithelial cells in the urine. A few epithelial cells will be present in every specimen. Generally, there will be more epithelial cells in specimens from females than in specimens from males. Partly because of the predominance of vaginal epithelial cells in many specimens, it is advisable to obtain "clean catch" specimens. These epithelial cells from the vagina are of the squamous variety and have no significance in the study of renal disease. Squamous cells are large and flat with round nuclei and may be cornified or uncornified. Ordinarily, no effort need be made to describe or classify them. If it becomes necessary to further describe epithelial cells, the Sternheimer-Malbin stain is very helpful.

White Blood Cells. Far more important than epithelial cells, from a clinical standpoint, are the white blood cells. A few leukocytes are present in normal urine, although it is for the physician to determine how many are normal. Increased numbers of leukocytes may originate from any part of the genital or urinary tract. When these cells are present in great numbers, especially neutrophiles, they are sometimes referred to as *pus cells*. There is no particular advantage in using this term, and instead the report should be given as the number of WBC's per hpf (high power field).

White blood cells are quite easily distinguished from red blood cells on the basis of size and the presence of internal structures. Obviously, white blood cells are larger, and unless accompanied by at least 5 or 6 red cells per hpf, will not give a positive benzidine test. Occasionally a white precipitate due to the presence of albumin will obscure the nuclear structure of the leukocytes. The addition of dilute acetic acid will clear the field and enable you to see the nuclei. It is important that red blood cells be identified before adding the dilute acid. Unless you do observe the nuclei, it is very difficult to distinguish white blood cells from rounded epithelial cells.

The presence of pus in urine is termed *pyuria*. In alkaline urine the leukocytes will adhere to each other in clumps. For an accurate count of those cells, the urine is acidified and a blood counting chamber is used. Qualitative or quantitative tests for albumin should be performed on the clear centrifuge which will be stressed again in the following chapter. It is often of diagnostic importance to know if proteinuria (protein in the urine) is occurring in addition to that derived from the pus cells themselves. For example, when pus cells are found without proteinuria, it is unlikely that the infection involves the renal parenchyma (functional tissue). The major causes of pyuria are tubular and pyogenic infections, tumors, and trauma.

Before concluding our discussion of epithelial cells and leukocytes, we will recognize two particular kinds of cells: the *oval fat body* and the *glitter cell*. Oval fat bodies are characteristic of degenerative tubular disease. They are found within the intact epithelial cell periphery as inclusions (fat droplets). Oval fat bodies may appear black under low illumination because of their high refractive index. They stain orange with Sudan III. Glitter cells are nonviable, polymorphonuclear leukocytes which contain many bacteria. They may be confused with epithelial cells that contain fat. Their name is derived from their glittering, shining appearance in moderately bright light. The diagnostic value of glitter cells is uncertain. They are reported to be of some diagnostic value in cases of pyelonephritis. With the Sternheimer-Malbin stain, glitter cells stain pale blue, whereas other white blood cells stain darker blue, as indicated in detail B of foldout 1. White blood cells, glitter cells, and oval fat bodies are also pictured in detail B.

Exercises (617):

Indicate whether the statement is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. It can be stated with certainty where in the urinary tract epithelial cells originate.

T F 2. "Renal" epithelial cells always come from the kidney.

T F 3. Large numbers of renal epithelial cells will be found in chronic kidney disorders.

T F 4. It is difficult to state the exact significance of epithelial cells in the urine.

T F 5. Increased numbers of leukocytes originating from any part of the genital or urinary tract, especially neutrophiles, are sometimes referred to as *ghost cells*.

T F 6. White blood cells may sometimes give a positive benzidine test when not accompanied by 5 to 6 red blood cells per hpf.

T F 7. The nuclear structure of the leukocytes may be obscured by the presence of albumin.

In questions 8 through 11, fill in the blank with the appropriate word or words. A phrase, rather than a single word, may be required for the correct response.

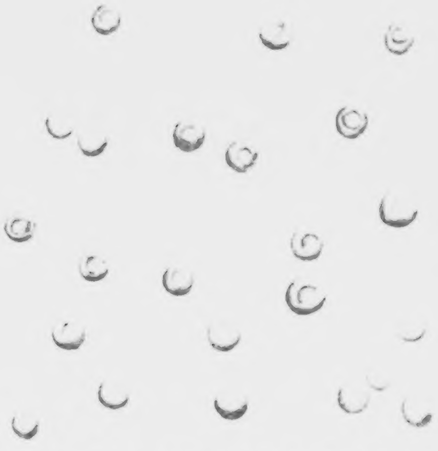
8. The presence of pus in urine is termed ________ .
9. When pus cells are found without proteinuria, it is unlikely that the infection involves the ________ .
10. Cells characteristic of degenerative tubular disease are known as ______ and appear black under low illumination because of their high refractive index.
11. Nonviable, polymorphonuclear leukocytes which contain many bacteria and stain pale blue with Sternheimer-Malbin are called ________ .

618. Define "hematuria", and state possible causes, factors that determine the appearance of the RBCs, methods for detection, and difference between forms found in hypertonic and hypotonic urine.

Hematuria. The appearance of blood in the urine, whether gross or only a few cells, is referred to as *hematuria.* Hematuria may be due to any one of several clinical conditions. These include carcinoma, chronic inflammation, renal calculi, nephritis, cystitis, and hemorrhagic diseases. In some cases, hematuria is the only reliable clinical symptom to aid the physician in diagnosis and treatment. You are probably already aware that hematuria occurs when incompatible blood is transfused into a patient. Trauma to the kidneys from an injury also produces bloody urine. In general, we can state that a significant amount of blood in the urine is a serious pathological finding unless it is the result of menstrual discharge. It is for the physician to determine how much blood is significant; however, a normal 24-hour urine specimen may contain up to 650,000 RBCs. This would represent only an occasional RBC per low power field (for example, one cell seen in 3 to 6 lpf). This value is the same for males and females but the possibility of contamination from the vagina or uterus of females is considerable. Urine that contains gross blood (discernible macroscopically) should be positive for protein.

Erythrocytes. The appearance of erythrocytes (RBCs) varies considerably depending upon the pH, specific gravity, and age of the specimen. Red cells may sometimes be confused with yeast cells or white blood cells. Actually, white blood cells are one and one-half times as large as red cells, and yeast cells frequently show budding. In addition to the microscopic method, chemical methods may be used to detect hematuria. If there is any doubt, add a drop of 10 percent acetic acid. Red blood cells will hemolyze, whereas other structures are unaffected. Other more current and reliable tools used in the chemical method include Hemastix, a reagent strip, or the hemoglobin area of Bili-Labstix and Occultest, a reagent tablet. Red cells may be intact, crenated (scalloped or notched), or ghost (hemolyzed). All three types are shown in figure 4-1.

Crenation of erythrocytes occurs in *hypertonic* urine, and hemolysis is due to swelling and bursting of the red cells in *hypotonic* urine. As you will recall from your study of clinical chemistry, a hypertonic solution is one in which the concentration of ions (other than water) is greater outside the cell than inside. A hypotonic solution is a solution in which the concentration of ions is less outside the cell than inside. Consequently, in a hypertonic medium water is withdrawn from the cells, and in a hypotonic medium water moves into the cell. Dilute urine is hypotonic, and as a result, *ghost* forms of erythrocytes may be observed. The urine in this case will still give a positive result in chemical tests which detect hemoglobin.



INTACT RED BLOOD CELLS

GHOST BLOOD CELLS

CRENATED RED BLOOD CELLS

Figure 4-1. Three types of RBCs.

Large amounts of blood that have been present in urine for any length of time will cause the color of the urine to be a smoky red-brown. However, bile or various dye substances which are excreted may also cause the urine to appear this color. Hence, color alone is not a reliable indication of blood. Fresh blood in urine appears bright red.

Exercises (618):

1. What is hematuria?

2. What are some clinical conditions causing hematuria?

3. The appearance of the RBCs varies considerably depending upon the ________ , ________ , and ________ of the specimen.

4. List the chemical methods for detection of hematuria.

5. Crenation of erythrocytes occurs in ______ urine.

6. What forms of erythrocytes might be observed in a dilute hypotonic urine?

619. Identify the various types of casts and related structures by their shape, size, formation, and staining characteristics.

Casts. Cylindrical bodies which are formed in the lumen of the renal tubules are referred to as *casts*. They usually consist of a hyaline matrix in which organized sediment is embedded. However, other types of casts may also be found on occasion. Casts are differentiated by their microscopic appearance (coarse, fine, or hyaline granules); identification of inclusions (epithelial, red cell, or bacterial); the chemical nature of their inclusions (waxy, fatty, or hyaline); and finally by their histochemical reactions or staining characteristics. These are overlapping distinctions, and you must keep this in mind as they are discussed.

TABLE 4-1
CLASSIFICATION OF CASTS IN URINE

A. *Hyaline*
  1. *Transudation (clear hyaline)*
  2. *Inclusion Casts*
    a. Fine Granular
    b. Coarse Granular
    c. Tubular Epithelial
    d. Red Cell
    e. Other—Bacterial, Crystalline, Fatty
B. *Epithelial*
  1. *Fresh Cell Cast*
  2. *Degenerated*
    a. Fine Granular
    b. Coarse Granular
    c. Waxy
    d. Fatty

Table 4-1 represents one classification of casts in urine. Let us discuss each of the types of casts mentioned in this table. Note that our system of classification does not follow that outlined in AFM 160-49, *Clinical Laboratory Procedures—Chemistry and Urinalysis*. The classification presented in this chapter is somewhat more systematic and based upon distinctions which can be discerned with the Sternheimer-Malbin stain.

If you will look at the listing in table 4-1 you will see two major categories of casts: A, Hyaline, and B, Epithelial. These distinctions are based upon the original material that formed the cast.

Hyaline. The *clear hyaline cast* is formed by the precipitation of protein in the lumen of the kidney tubules. Hyaline casts rapidly disappear in an alkaline medium. A hyaline cast consists exclusively of a colorless, slightly refractile matrix. Consequently, it has the appearance of almost complete transparency. If stained, it appears pink to light purple and has a homogenous central portion. Hyaline casts are sometimes called transudation casts. A clear hyaline cast is illustrated in detail D of foldout 1.

In addition to clear hyaline casts there are various types of inclusion casts under the broad classification of hyaline (see table 4-1). Practically any cellular material that is present in the kidney tubules may be trapped in the precipitated matrix of a hyaline cast. The casts may appear finely or coarsely granular. Granules stain purple, and the hyalin matrix stains pink. The cellular source of the granules is uncertain. They may be degenerated blood cells or epithelial cells. If the tubular epithelial cells are intact within the hyalin matrix, we refer to the cast as a *tubular epithelial inclusion* cast. The epithelial cells are from the renal tubules and represent a pathological process. A *tubular epithelial* cell inclusion cast is shown in detail C of foldout 1.

Refer again to table 4-1. Under A.2, *hyaline inclusion* is the red cell of inclusion cast. Unstained red blood cells casts are usually orange in color. If the cast appears homogenous without defined cellular outlines, it is referred to as a *blood cast* rather than a *red cell cast*. Some evidence suggests that clinical value can be derived from identifying these two kinds of casts. Blood casts are rarely seen unless associated with glomerulitis or, in some cases, with collagenic disorders. Red blood cell casts occur with greater frequency in many other conditions. A red cell inclusion cast appears as a mass of red blood cells which stain pale lavender. The cells are usually tightly packed, so that little of the pink staining matrix can be seen. Graduations of the two types may be found. Both the blood cast and the red cell cast are compared in detail E of foldout 1.

The final listing in table 4-1 under hyaline inclusion cast includes bacterial, crystalline, and fatty hyaline inclusion casts. Bacteria within casts stain dark purple and can usually be identified as bacteria without difficulty. Occasionally bacteria may be seen within the white blood cells, giving rise to *glitter cell casts*. Crystals can be identified within casts by their characteristic geometric design. Ordinarily, it is not necessary to identify the cast beyond the fact that it is a *crystal* inclusion cast. A fatty inclusion cast incorporates highly refractive fat droplets. Fat will not stain with the Sternheimer stain. Fatty casts are characteristic of degenerative tubular disease.

Epithelial. The second major classification of casts in table 4-1 is B, *Epithelial.* Epithelial casts, in contrast to hyaline casts, are formed by desquamated epithelial cells which are not cemented by a hyalin matrix. Rows of cells may slough off the kidney tubules, suggesting considerable damage to the tubule lining. The mold of the kidney tubule, itself, shapes the cast. If this type of cast appears with distinct cell margins, it may be referred to as a *fresh tubular epithelial cell cast* (see detail C of foldout 1). However, an epithelial cell cast of this type does not always remain intact. It may degenerate to form a *coarse granular cast or a fine granular cast.* These two kinds of casts are illustrated in detail F of foldout 1. There is no hyalin material present which can be detected with the Sternheimer-Malbin stain. If the cast disintegrates further until a homogenous mass is formed, it then becomes a *waxy cast.* Detail F of foldout 1 also shows a waxy cast. A waxy cast is the most degenerate form of epithelial cell cast. It stains a uniformly dark purple. This is due to the inclusion of amyloid, an abnormal protein which deposits in the kidney. The waxy cast is the most likely to be found in urine of high specific gravity following a period of oliguria. Waxy casts are found in cases of severe renal lesions and in some cases in amyloid degeneration and advanced nephritis.

Formation, shape, and size. The size of casts is a consideration worth noting. Of course, a cast from a child will be smaller than a cast from an adult because the lumina of the tubules in a child's kidney are smaller. With due regard to this expected difference, casts are occasionally seen which are very narrow. This is significant because it suggests a swollen tubular epithelium. Conversely, very broad casts may be observed. Their presence is an important laboratory finding. *Broad casts* are formed in the large collecting tubules and result from urinary stasis which, in turn, is caused by the diminished function of large numbers of nephron units. Although they are usually granular, broad casts may be any of the types previously discussed and are associated with renal failure.

It is important to recognize that cast formation is generally described in terms of theories. Consequently, it is difficult to classify casts without subscribing to a particular theory of how casts are formed. One theory suggests that waxy casts and fatty casts are derived from hyaline casts; and that epithelial casts are merely one type of hyaline cast. More recently, some authorities have identified two types of epithelial and granular casts, one being hyaline, and the other nonhyaline. This distinction was made in the preceding discussion because there is a type of granular cast in which a hyalin (albuminoid) matrix cannot be demonstrated. It is assumed that this latter type of granular cast is derived from epithelial casts which have either lost their hyalin matrix or never contained a hyalin matrix. These casts may contain protein, but not the hyalin material which is characteristic of hyaline casts. The need to distinguish the two categories of granular casts (granular hyaline inclusion and granular desquamation casts) is still uncertain. Actually, the distinction is easy enough to make based on the respective histochemical reactions with the Sternheimer-Malbin stain. Hyalin stains bright pink. Epithelial cell casts without a hyalin matrix are desquamation casts. Epithelial casts with a hyalin matrix are inclusion casts. This distinction may be of some value to the clinician.

Cylindroids resemble casts, but usually have one drawn-out tapered end. They are of no great clinical significance other than being present in increased numbers in inflammatory conditions. Their origin is not known. Mucous threads are long, slender, transparent strands which may normally be found in small numbers. Increased numbers are present in urethra and bladder irritations. They are usually twisted into various shapes with a tapered end as shown in figure 4-2, and this characteristic aids in distinguishing them from casts.

Spermatozoa are easily identified by their characteristic shape, but their presence is generally not reported unless specifically requested by the physician. Yeast cells may be present pathologically or as contaminants. These cells are sometimes confused with red cells. A careful study of the specimen will usually reveal budding, which is a characteristic of yeast cells as suggested earlier and illustrated in figure 4-3. Parasites are sometimes found in urine, too. The most common are *Trichomonas* species. *Schistosoma haematobium* is less common but may be found in the urine. A discussion of these parasites will be found in Career Development Course 90412.

Exercises (619):

Match the casts and related urinary sediment in column B with the features and characteristics in column A by placing the letter of the column B item beside the number of the column A item. Each column B item may be used once or more than once.

Column A

____ 1. Formed by the precipitation of protein in the lumen of the kidney tubules.
____ 2. Rapidly disappear in an alkaline medium.
____ 3. Tubular epithelial cells intact within the hyalin matrix.
____ 4. Cells of casts are so tightly packed that little of the matrix can be seen.
____ 5. Bacteria within white blood cells result in this type of cast.
____ 6. Characteristic of degenerative tubular disease.
____ 7. Incorporates highly refractive fat droplets.
____ 8. Formed by desquamated epithelial cells

Column B

a. Fresh tubular epithelial casts.
b. Waxy casts.
c. Broad casts.
d. Granular hyaline inclusion and granular desquamation casts.
e. Cylindroids.
f. *Trichomonas* species.
g. Clear hyaline casts.
h. Tubular epithelial inclusion casts.
i. Red cell casts.
j. Glitter cell casts.
k. Fatty casts.
l. Epithelial casts.
m. *Schistosoma haematobium*

Figure 4-2. Cylindroids.

Figure 4-3. Yeast cells.

Column A	*Column B*
which are not cemented by a hyalin matrix.	
____ 9. This type appears with distinct cell margins and is formed from the tubule matrix.	
____ 10. The most degenerate form of an epithelial cell cast; stains a uniformly dark purple.	
____ 11. Most likely found in urine of high specific gravity following a period of oliguria.	
____ 12. Formed in the large collecting tubules and result from urinary stasis.	
____ 13. Distinction can be made on the respective histochemical reaction with the Sternheimer-Malbin stain.	
____ 14. Resemble casts, but usually have one drawn-out tapered end.	
____ 15. The most common parasite found in urine.	

4-2. Crystalline and Amorphous Sediments

The majority of the crystals found in fresh urine are not clinically significant. If present in greatly increased numbers, however, they may be important. In addition, certain pathologic conditions are accompanied by the excretion of abnormal crystals in the urine. Learn to identify the common types and the most common clinically significant crystals. In doubtful cases it may be advisable to consult the physician and inquire about medication and other pertinent facts which may have produced the crystals in question.

620. Indicate whether given statements correctly reflect the influence of urine pH on precipitation, solubility, and appearance of normal and amorphous crystals.

Influence of pH. An approximate classification of inorganized sediments based upon the pH of the urine may be used, but this division is not absolute. For instance, some crystals normally found in acid urine may be present in a sample which is neutral or slightly alkaline. The change from acid to alkaline urine crystals occurs gradually as the acid constituents deteriorate. Upon aging of the specimen, the number of crystals increases. Since most inorganic substances are more soluble at higher temperatures, cooling of urine will cause precipitation of crystals. In some cases it may be necessary to gently warm specimens taken from the refrigerator or change the pH in order to obtain a clear microscopic field. Heavy amorphous sediment (without observable structure) will make it very difficult to accurately perform a microscopic analysis.

"Normal" crystal and amorphous content of alkaline urine. Triple phosphate (ammonium magnesium phosphate), dicalcium phosphate, amorphous phosphates, and ammonium biurate crystals are frequently found in alkaline specimens. These crystals are all soluble in acetic acid and as a group may be differentiated from other crystals by this characteristic.

Triple phosphate crystals are colorless, highly refractile prisms, varying in size and presenting three, four, or six sides, giving the typical coffin-lid forms. The edges will often appear colored. Dicalcium phosphate usually crystallizes near the neutral point (pH 7.0), forming slender, colorless prisms with one pointed end. Various forms of triple phosphate crystals are given in figure 4-4. Amorphous phosphates are common in alkaline urine and appear as a granular precipitate.

Ammonium biurates are precipitated only when free ammonia is present. They may be found in several different forms, for example, in sheaves of fine needles and dumbbells as pictured in figure 4-5. They have a characteristic yellow color, and dissolve when the urine is warmed. Ammonia is liberated upon the addition of acetic acid.

"Normal" crystal and amorphous content of acid urine. Uric acid, amorphous urates, and calcium oxalate crystals are often found in acid specimens,

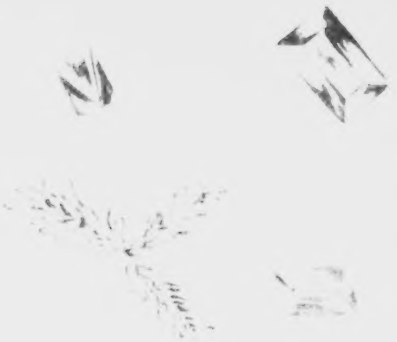


Figure 4-4. Triple phosphate crystals.

particularly after standing. Uric acid crystals are found in many different forms. The rhombic shape with an occasional rosette, as seen in figure 4-6, is typical. Uric acid crystals are soluble in sodium hydroxide. They are typically yellow or red-brown in color and may, like urates, give a cloudy or milky appearance to the specimen.

Amorphous urates are common and appear as a granular precipitate, usually pigmented. Differentiation between this type of urates and amorphous phosphates may be made by noting the pH of the urine, the effect of 10 percent sodium hydroxide, or gentle heating of the urine. Amorphous urates are soluble in alkali and upon warming.

Calcium oxalate crystals are most frequently found in acid urine but may also appear in neutral and alkaline urine. They are of little importance, and usually result from a diet rich in tomatoes, rhubarb, and asparagus. Calcium oxalate crystals vary greatly in size and shape but are generally seen as colorless, octahedral (box-shaped) crystals resembling small squares crossed by two intersecting diagonal lines ("envelope" appearance) as pictured in figure 4-7. They may also appear as dumbbells or spheres.

Figure 4-5. Ammonium biurates.

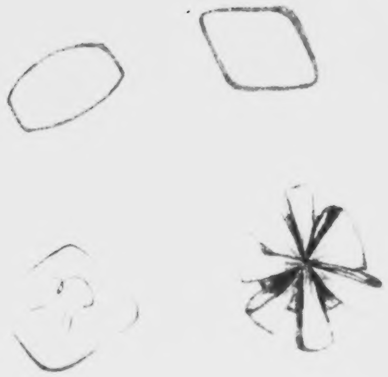


Figure 4-6. Uric acid.

Exercises (620):

Indicate whether each sentence is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. Some crystals normally found in acid urine may be present in a sample which is neutral or slightly alkaline.

T F 2. Triple phosphate, amorphous phosphates, and ammonium biurate crystals are all insoluble in acetic acid.

Figure 4-7. Calcium oxalate.

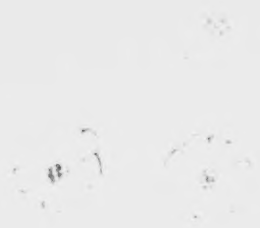


Figure 4-8. Leucine.

T F 3. Triple phosphate crystals are colorless, highly refractile prisms, typically rhombic shaped.

T F 4. Ammonium biurates are precipitated only when free ammonia is present.

T F 5. Uric acid, amorphous urates, and calcium oxalate crystals are often found in acid specimens.

T F 6. Uric acid is soluble in sodium hydroxide.

T F 7. Amorphous urates are insoluble in alkali and upon warming.

T F 8. Calcium oxalate crystals are most frequently found in acid urine, but are seldom found in neutral and alkaline urine.

T F 9. Calcium oxalate crystals are soluble in hydrochloric acid but not in acetic acid.

T F 10. Calcium oxalate crystals may also appear as dumbbells or spheres.

621. Identify abnormal crystals by distinctive shape and size and cite techniques of detection.

Abnormal Crystals. It is possible that you will find crystals of some of the amino acids or crystals of cholesterol in urine. The three amino acids we will consider are leucine, tyrosine, and cystine.

Leucine and tyrosine crystals are cleavage products of protein and usually occur simultaneously. They are formed as a result of serious liver damage, but they are rarely found in urine. Leucine crystals are yellow spheres often possessing radial and concentric striations, as illustrated in figure 4-8. They are soluble in alkali, but not in dilute hydrochloric acid or in dilute acetic acid at room temperature. Tyrosine crystals appear as very fine needles usually arranged in sheaves with a constriction in the middle as sketched in figure 4-9. Tyrosine crystals show the same solubility pattern just described for leucine, except that tyrosine is not soluble in boiling acetic acid, whereas leucine dissolves in this solvent.

There are rather simple chemical tests for tyrosine and leucine. To test for tyrosine, adjust the pH of an aliquot of the urine to be tested to 5.8. Then place the sample in the refrigerator until crystals are formed and can be separated. To this crystalline precipitate, add 2 ml of Morner reagent (Morner reagent: 1 ml formalin, 45 ml of water, 55 ml sulfuric acid added in this order). The mixture is heated to boiling and observed for the appearance of a green color which is considered a positive test for tyrosine.

To test for leucine, adjust the pH of an aliquot of urine to 6.8 or 7. Allow crystals to precipitate in the refrigerator. Then dissolve the precipitate in a few milliliters of water and add 1 drop of 10 percent copper sulfate. Leucine gives a blue color which remains stable when the mixture is heated.

Cystine is also a breakdown product of protein that

Figure 4-9. Tyrosine.

Figure 4-10. Cystine

appears very rarely. The crystals occur in acid urine as colorless, highly refractile, hexagonal plates with well-defined edges as shown in figure 4-10. Cystine crystals are not soluble in acetic acid; however, they are soluble in hydrochloric acid and in alkali. Crystals that have been separated may be identified by the Sullivan test. In this test, crystals are dissolved in 2 ml of water to which a few drops of sodium hydroxide have been added. Add 1 ml of 5 percent sodium cyanide solution and allow the mixture to stand for 10 minutes. In rapid succession, add the following: 0.5 ml freshly prepared 0.5 percent 1, 2 naphthoquinone-4-sodium sulfonate and 2.5 ml 10 percent anhydrous sodium sulfite in 0.2N sodium sulfite. After 30 minutes add 0.5 ml 2 percent sodium hyposulfite in 0.2N sodium hydroxide. The appearance of a red color is positive for cystine.

Cholesterol crystals are rarely found. They have a characteristic "missing corner" and appear in acid specimens as large, flat, colorless plates as sketched in figure 4-11. Other rarely found crystals include calcium sulfate, hippuric acid, hematoidin, indigo, and fatty acids.

Sulfonamides. Following treatment of the patient with sulfa drugs, crystals of the sulfonamides and their derivatives may be found in either alkaline or acid urine. Conjugated derivatives; for example, acetylsulfathiazole; are less likely to be found in alkaline urine. Sulfamerazine and sulfadiazine are also less likely to be found in alkaline urine because of their increased solubility at higher pH levels. Unfortunately, the crystals which you will observe are often derivatives of the drug that was administered. Consequently, the shape of the crystals will vary considerably. Free sulfonamides and acetylsulfanilamide are less likely to be found than other derivatives because of their relatively high solubility.

Figure 4-11. Cholesterol.

Exercises (621):

Match the abnormal crystals in column B with distinctive characteristics in column A by placing the letter of the column B item beside the number of the column A item. Each column B item may be used once or more than once.

Column A

____ 1. May be found in either alkaline or urine following treatment of patient with sulfa drugs.

____ 2. Yellow spheres often possessing radial and concentric striations.

____ 3. Appear as very fine needles usually arranged in sheaves with a constriction in the middle.

____ 4. pH is adjusted to 5.8 and specimen placed in refrigerator until crystals are formed and can be separated.

____ 5. The crystals occur in acid urine as colorless, highly refractile, hexagonal plates with well-defined edges.

____ 6. To test for these crystals urine pH is initially adjusted to 6.8 or 7.

____ 7. Have a characteristic missing corner and appear in acid specimens as large, flat, colorless plates.

____ 8. Less likely to be found in alkaline urine because of their increased solubility at higher pH levels.

Column B

a. Leucine.
b. Tyrosine.
c. Cystine.
d. Cholesterol crystals.
e. Sulfonamides.
f. Sulfamerazine.
g. Sulfadiazine.

CHAPTER 5

Chemical Examination

CHAPTER 2 OF this volume covered the basic physiology of the renal system. You may wish to refer to Chapter 2 as we begin a description of qualitative urine chemical tests. You should recall that the quantitation of some urinary chemical constituents and their physiological significance have previously been studied in Volumes 2 and 3 of this course. In these preceding volumes on blood chemistry, where the chemical constituent was common to both blood and urine, the quantitative aspects of urine chemistry were also discussed. For example, the chapter on proteins of Volume 2 covered chemical and metabolic aspects of protein excretion. The physiological chemistry of liver function also included consideration of bile pigments and the physiological mechanism by which they appear in urine. Kidney function tests were covered at length in Volume 3.

This chapter deals with the technical aspects of screening urine specimens (qualitative analysis) for various biochemicals of clinical importance and, when necessary, roughly estimating the quantity of a chemical constituent (semiquantitative analysis). As you study this chapter, keep in mind that although the methods used are the simplest in the clinical laboratory, the physiological mechanisms which you are measuring involve very complex biochemical principles.

5-1. Urinary Protein

Normally an amount of protein between 40 and 80 mg is excreted per day, but as much as 100 to 150 mg per day may be considered within normal limits. The following paragraphs discuss the nature of urinary proteins and methods of detection.

622. Point out the procedures for urine protein screening in terms of origin of the proteins, causes of proteinuria, confirmatory testing, protein fractions, and tests.

Nature of Urinary Proteins. A qualitative test for protein in urine (proteinuria) is one screening test always included in routine urinalysis. Healthy adults normally excrete less than 100 gm of protein in 24 hours. This protein excreted by the kidneys has been shown to be derived from the plasma proteins. Although albumin is the primary protein fraction normally excreted, smaller quantities of globulin are also eliminated. It is theorized that more albumin is excreted by the kidneys because of the relatively low molecular weight (70,000) of albumin and its correspondingly smaller molecule. Normally, the glomerular filter is impermeable to protein molecules larger than albumin, that is, globulin (MW 165,000).

In active renal disease, protein-like substances called proteoses have been indentified in urine by electrophoresis. Proteoses migrate with the globulins electrophoretically and interfere with some tests for protein. Protein is also contributed to urine from the lower genitourinary tract. This contribution includes protein from the ureters, bladder, urethra, and prostate. These constituents are nonserum proteins with an electrophoretic mobility slightly greater than that of albumin. They are the so-called "Tamm-Horsfall" mucoproteins and are important because of their confusion with serum-derived protein.

Transitory proteinuria may result from extraordinary physical exercise or ingestion of a high protein meal. The temporary impairment of renal circulation which results from postural aberration may also cause a transitory proteinuria. This impairment is known as orthostatic proteinuria, and it disappears when the impairment is relieved. Proteinuria in these cases cannot be attributed directly to kidney disease. It is usually temporary and does not exceed 500 mg/day. Proteinuria may also occur in disease states that are not primarily renal, such as febrile conditions. Another extrarenal cause of albuminuria is bleeding into the urogenital tract as a result of trauma or disease. This could result from trauma during the passage of urinary calculi, for example.

The critical importance of testing for proteinuria, however, is in the diagnosis of kidney disorders. It has been said that proteinuria is probably the best single indication of renal disease. For this reason, proteinuria is always thoroughly evaluated by an attending physician. Consequently, most authorities recommend that all positive urine protein screening tests be confirmed by a second and different qualitative test. Confirmatory testing in this instance is done for two reasons: (1) the clinical importance of the result and (2) the limitations of any single method.

Urine Protein Methods. Probably because of the long history of urinalysis and its time-honored place in clinical diagnosis, many methods exist for the qualitative analysis of proteinuria. Although older texts and some newer revisions refer to *albuminuria,* it is a misnomer. All qualitative analyses used in urinalysis identify protein and are not specific tests for the albumin fraction. Some reagents used in testing for proteinuria are more selective than others. You may recall that in Volume 2 of this course we spoke of various precipitants for protein fractions. You should remember also that these reactions are dependent upon the nature of the protein (that is, isoelectric point) and the nature of the reagents (that is, pH and salt concentration). These principles are also important to remember in studying the qualitative analysis of urinary proteins.

A complete listing of all the known urine protein screening tests is impractical. These tests involve the use of heat and acetic acid, sulfosalicylic acid, trichloroacetic acid, picric acid, nitric acid, and biuret reagent (used alone or in combination with salts such as sodium chloride, magnesium sulfate, potassium ferrocyanide, and sodium acetate). The literature is filled with these methods and more. Some use salts in spot tests, in dilute and concentrated solutions, and as test tube ring tests.

The more modern approach is to use a buffer salt with an indicator which changes color at different protein concentrations or a colorimetric reagent strip test. Such a reagent has been incorporated into a well-known test strip for rapid screening of urine specimens. This procedure will be discussed in more detail later in this chapter. Because of the necessity for a confirmatory test, several other methods will also be included in our discussion. The heat with acetic acid technique and sulfosalicylic acid test are probably the most widely used of these other tests.

Exercises (622):

1. Where is protein excreted by the kidneys derived?

2. What nonserum proteins are contributed to urine?

3. What is orthostatic proteinuria?

4. List three causes of proteinuria which are not directly related to kidney disease.

5. What are two reasons for confirmatory testing of urine protein screening tests?

6. What protein fraction is specifically measured by qualitative urine screening tests?

7. What are three most widely used urine protein screening tests?

623. Identify the basic prerequisites for qualitative screening tests for urine protein in terms of normal ranges, sensitivity of tests, and difficulty in using the biuret reaction.

Prerequisites. At this point we might consider what prerequisites are desirable in a qualitative screening test for urine protein. First of all, the test must detect *abnormal* amounts of protein. In order to define an abnormal amount, we must first decide upon the so-called "normal" limits. Since we are concerned with a *screening* test, we will be interested primarily in only the upper normal limit. Ranges in the literature for normal 24-hour urine protein extend from a low of 20 mg/24 hours to a high of 250 mg/24 hours. This wide range is understandable in view of variables which influence this value, such as 24-hour urine volume, diet, method of analysis, and normal individual physiological fluctuations. It is obvious that clinical interpretation by a physician is essential to determine any specific norm for a particular individual.

Considering all the above factors, current literature has an upper limit of established "normal" between 100 and 150 mg/24 hours. If normal fluctuations in urine volume are accounted for, this amounts to 7 to 10 mg/100 ml of urine. Since the method employed is important, it should be noted that these analyses used trichloroacetic acid precipitation in two cases with the biuret color reaction for the lower value (20 to 75 mg/24 hours) and photoelectric measurement of turbidity for the higher value (20 to 100 mg/24 hours). Precipitation with alcohol and reaction with biuret yielded the lowest value of "at least 71 mg/24 hours." It was demonstrated electrophoretically that alcohol does not precipitate most of the proteoses.

These figures establish another criterion for a qualitative urine protein test. The test should not be so sensitive that it will react with "normal" amounts of urine protein. In other words, the sensitivity should be no greater than 5 to 10 mg/100 ml of urine. Under certain conditions, trichloroacetic acid and sulfosalicylic acid will detect protein concentrations of less than 1 mg/100 ml. Consequently, under such itions. these tests would be unacceptable for ning urine protein. The problem of excessive itivity can be eliminated with appropriate dilution ie protein specimen. The biuret reaction which you ied in Volume 2 is also extremely sensitive to te protein solutions. The difficulty in using biuret screening test arises from its nonspecific reactions i many other normal urine constituents.

cises (623):

ist three factors that influence the normal range t protein in a 24-hour urine specimen.

2. Give the upper limits for normal urine protein in gm/100 ml.

3. Why is the sensitivity of a urine protein screening test important?

4. Why is the use of buiret unsuitable as a screening test for urine proteins?

TABLE 5-1
POSSIBLE FALSE REACTIONS IN QUALITATIVE URINE PROTEIN TEST

Causable Agent	Sulfosalicylic Acid (Exton's Reagent)	Nitric Acid (Heller)	Heat & Acetic	Heat & Buffered Acetic	Reagent Strip
Urine Turbidity[1]	±	±	±	±	
Tolbutamide[2] Metabolites (Orinase)	+	+	+		
X-ray Media Iodine	+	+	+		
Penicillin (Massive doses)	+	+	+		
Sulfa (Gantrisin)	+	+	+		
PAS[3]	+	+	+		
Strong Alk. Buffer	-		-		+
Proteoses	+	+	+	+	
Mucin		+			
Quaternary Ammonium Salts					+
Resinous Acids[4]		+	+	+	
Urea		+			
Uric Acid	+	+			
Strong Dye					±
Reagent Contamination					±
Leaching Reagent					-

1. May be removed by centrifugation
2. Used in treatment of diabetics
3. Para-amino-salicylic Acid
4. From drugs such as benzoin

TABLE 5-2
POSSIBLE REACTIONS OF URINE AND ACETIC ACID IN THE QUALITATIVE SCREENING TEST FOR PROTEIN

Specimen Appearance	After Boiling	After Acidifying	After Reboiling	Interpretation
Clear	Clear	Clear		Normal*
Clear	Cloudy	Clear		Phosphates
Clear	Clear or Cloudy	Clear Effervesces		Carbonates
Clear or Cloudy	Below 60°C. Cloudy	Cloudy	Over 60°C. Clear	Bence-Jones or Proteoses
Clear	Cloudy	Sl. Cloudy to Flocculent	Same or Increase Cloud	Positive** Protein
Cloudy	Clear	Clear		Urates
Cloudy	Clear	Clear	More Cloudy	Excess Urates
Cloudy	Cloudy	Cloudy	Cloudy	Microorganisms***

* Normal Protein Less Than 5 to 10 mg/100 ml.
** Positive Protein Greater Than 5 to 10 mg/100 ml.
***Microorganisms Confirmed Microscopically

624. Identify the possible causes of false reactions in protein screening tests and techniques for eliminating these reactions.

False Reactions. False reactions have always been a problem with urine screening tests. As stated previously, erroneous reactions are the prime reason for confirmatory testing. Look at table 5-1 and you will see some of the possible causes for false reactions reported in the more common protein screening tests. It is evident from the table that the nitric acid ring test reacts with many substances found in normal urine. The excessive number of erroneous reactions renders this test practically worthless as a protein screening test. The Roberts (1 part concentrated nitric acid added to 5 parts saturated aqueous magnesium sulfate) modification is said to have fewer nonspecific reactions, but research support for this premise is lacking.

It should also be stated that the false reactions listed are not invariable. For instance, penicillin "in massive doses" is reported to interfere with both the nitric acid and sulfosalicylic acid tests with no qualification as to a more exact concentration. Further qualification in the legend of table 5-1 should be noted. As it indicates, urine turbidity can be discounted entirely as the cause of erroneous results if the urine can be cleared by centrifugation. You are aware that clearing by centrifugation can be done with many specimens, and that centrifugation is the usual procedure when a microscopic examination is requested.

If proteoses are interfering, they can be dissolved in saturated picric acid, which also precipitates protein. Thus, in this case a subsequent test specific for the interfering substances may also be used to substantiate results. Modifications in method, such as the use of heat with sulfosalicylic acid, can be used to dissolve proteoses and Bence-Jones protein without affecting precipitated albumin and globulin. These dissolved substances can be identified by clearing of the specimen when it is warmed and thereby excluded as a cause of false reactions.

Subjective interpretation by the technician, although undesirable technically, is unavoidable with these qualitative tests. In table 5-2 the listing of reactions observed with heat and acetic acid at various points in the procedure clearly illustrates the interpretation required of a technician. Without a thorough understanding of these reactions, valid results are not attainable.

Even the widely used reagent strips are subject to false reactions. Highly buffered alkaline urine which may be encountered in aged specimens gives a falsely positive color reaction. This reaction is noted in table

5-1. The reason is that the strip contains a buffer salt which maintains a pH of 3, and the indicator will indicate protein concentration accurately only at this pH. Any substance that breaks this buffer system will invalidate the reaction. This principle is detailed in the discussion of reagent strips. If the buffer salt deteriorates through improper storage or is contaminated in handling, the buffer system could be destroyed. Any strongly alkaline reaction with a positive protein strip test should be checked with heat and acetic acid.

As shown in table 5-2, the heat and acetic acid test may give a false *negative* reaction in highly buffered alkaline urine. If it is *positive*, however, it is a valid positive protein test. A false reaction is less likely to occur if the acetic acid contains buffer salts, as mentioned in the following section on heat and acetic acid. One last factor, which involves only the reagent strip test for protein, is the matter of highly colored urine. Such specimens are occasionally encountered, and since the reacted reagent strip presents a color reaction, strong chromogens may confuse this color differentiation. In the presence of protein the color will change to green and then to blue with increasing protein concentrations.

It must be obvious at this point that no single qualitative test for protein is perfect. Let us review the prerequisites for a qualitative urine protein screening test. First, it should be sensitive enough to detect all abnormally high concentrations of urine protein, but not so sensitive that it reacts with normal urine protein concentrations. Second, it should have few, if any, false positive or negative reactions. However, since no protein screening test is without false reactions, you as a technician should be aware of the reactions that may occur. Then you should know what alternate screening test will verify the true protein reaction. The stability of reagents used for the test should be included among the prerequisites. Finally, the number of urine specimens processed in most laboratories demands an efficient operation, and the medical staff naturally insists on technical competence. You must select the screening test or tests which are both efficient and appropriate.

Exercises (624):

Match each item in column B with the appropriate reaction or technique to which it closely relates in column A. Each item in column B may be used once or more than once or not at all.

Column A	*Column B*
____ 1. May cause a false positive color reaction with reagent strips.	a. Penicillin. b. Proteoses. c. Highly buffered alkaline urine.
____ 2. False reactions with sulfosalicylic acid, nitric acid, heat, and acetic acid tests.	d. Quaternary ammonium salts. e. Modifications—heat and sulfosalicylic acid.
____ 3. Can be dissolved in saturated picric acid if identified as an interfering substance.	f. Nitric acid and sulfosalicylic acid. g. Heat and acetic acid test. h. Reagent strip.
____ 4. Can be used to dissolve proteoses and Bence-Jones protein without affecting precipitated albumin and globulin.	
____ 5. Should be checked with heat and acetic acid.	
____ 6. May give a false *negative* reaction in highly buffered alkaline urine.	
____ 7. Strong chromogens may confuse color differentiation.	

625. Indicate whether given statements correctly reflect the procedures for the heat and acetic acid test for urine protein.

Heat and Acetic Acid Test. The heat and acetic acid test has been mentioned several times as a screening test for urinary protein. The principle of the test is simply that urinary protein will coagulate when heated at an optimal acid pH. Several modifications have been used. The method used for the reactions obtained in table 5-2 began with 2 to 3 ml of unfiltered urine. The urine was first boiled thoroughly, then acidified with 2 to 3 drops of 50 percent (V/V) acetic acid, and boiled again. The readings were observed as indicated in table 5-2.

A modification of the heat and acetic acid method uses 10 to 15 ml of centrifuged, clear urine in a pyrex test tube. Centrifugation removes most crystals and cellular components which give a cloudy appearance. After clearing, heat the upper portion of the specimen to boiling. At this point, if the upper portion remains clear, the urine is negative for protein. If it is cloudy, it is acidified with 2 to 3 drops of glacial acetic acid and reboiled. A persistent cloud after reboiling is a positive reaction of protein. Study the list of reactions again in table 5-2 to be sure you understand the sequence of reactions which represent both positive and false reactions with this method.

The purpose of boiling the specimen is to drive off carbon dioxide (CO_2), lowering the buffer capacity, which in turn will make the normally acid urine even more acid. The optimal pH for protein precipitation is between 4 and 5. However, you will notice in table 5-2 that phosphates and carbonates as well as protein may precipitate after this initial boiling. Therefore, acetic acid is added to dissolve phosphates and react with carbonates, which go off as gas, leaving protein and perhaps excess urates. You can imagine that a highly buffered, alkaline urine may prevent the urine from becoming acid enough to precipitate protein. This results in a false negative reaction for urine protein.

More recently it has been proposed that sodium acetate be added to the acetic acid to form an acid reagent buffered at pH 4. This reagent would tend to stabilize the reaction at an optimal pH for protein precipitation. As you can see in table 5-1, however, proteoses and resinous acids (from drugs) will react even in the buffered acid reagent. Although sulfa-para-amino salicylic acid and high concentrations of penicillin have been reported to interfere with the heat and acetic acid without buffer, they do not apparently cause false positive reactions with the buffered acid reagent.

Exercises (625):

Indicate whether each of the following sentences is true (T) or false (F).

T F 1. Centrifugation removes most of the crystals and cellular components in the urine specimens for heat and acetic acid tests so that they will not interfere with the reaction.

T F 2. Persistent cloudiness of an originally clear urine after acidifying, which increases with reboiling, is a positive reaction for microorganisms.

T F 3. The optimal pH for protein precipitation is between 4 and 5.

T F 4. A highly buffered, alkaline urine may result in a false negative reaction for urine protein.

T F 5. Sulfa-para-amino salicylic acid and high concentrations of penicillin will apparently cause false positive reactions with the buffered acid reagent.

626. Specify the procedures for the reagent strip test for urine protein by citing the principle, methods, and interpretation of results.

Reagent Strip. The reagent strip test for protein is based upon a rather time-honored principle. In 1909, Sorensen noticed that certain pH indicators turn different colors at the same pH in the presence of protein. He called this phenomenon the "protein error of indicators." In 1957, this principle was used commercially to prepare a powder mixture of bromphenol blue indicator and a salicylate buffer (pH 3) in tablet form. In tests with urine specimens positive for protein, it was discovered that the concentration of protein in the specimen affects the intensity of the blue-green positive reaction. Subsequently, an absorbent paper strip was impregnated with tetrabromphenol blue indicator and a citrate buffer at pH 3. The negative indicator color is yellow with a range of positive colors from light green to aqua blue. These colors are illustrated on the multideterminant reagent strips, COMBSTIX®, LABSTIX®, BILI-LABSTIX®, and MULTISTIX® available through standard or local purchase supply channels.

A plastic strip with attached porous paper squar used to facilitate drainage when the reagent stri dipped in urine. The nonwettable plastic prevents excess of specimen from being absorbed by the reag squares. As stated previously in Chapter 4 of t volume, the reagent strip must not be dropped into t specimen. Follow the directions as they are given the reagent bottle.

Protein is determined simply by dipping the str into well-mixed uncentrifuged urine, and immediate comparing the resultant color with the chart provid on the reagent strip bottle. The results are reported negative (yellow color), trace, or one "plus" to fo "plus." Trace readings may detect 5 to 20 mg protein per 100 ml. Positive or "plus" readings ar approximately equivalent to protein concentrations 30, 100, 300, and 1000 mg per 100 ml, respectively and are reliable indicators of increasingly sever proteinuria. It should be noted that albumin react more strongly than do other proteins.

In view of possible false positive reactions with strongly alkaline urine, you should consider the pH and protein portions together when you read a protein reaction. A pH above 8 with a positive protein should be reevaluated with another test for protein—that is, heat and acetic acid.

Exercises (626):

Complete each sentence with the appropriate word or words. A phrase, rather than a single word may be required for the correct response.

1. Certain indicators, such as tetrabromphenol blue, will turn different colors at the same pH in the presence of __________.
2. The ideal (optimum) pH for protein reaction with the reagent strip is __________.
3. Trace readings may detect ______ to ______ mg of protein per 100 ml.
4. A one "plus" is equivalent approximately to ______ mg per 100 ml.
5. In view of false positive reactions, you should consider the ______ and ______ portions together when you read a protein reaction.
6. Pertaining to the indicator reactions, ______ reacts more strongly than do other proteins.

5-2. Urine Glucose

As stated earlier, tests for glucose in urine are also determined in all routine urinalyses. Screening urine specimens for glucose is the best method of detecting diabetes mellitus. It is also of critical importance to the physician in treatment of the diabetic patient. When you consider that diabetes afflicts approximately 5,000 persons per million of population, the importance of detection and treatment becomes even more apparent. Often glucosuria does not occur until the blood glucose is considerably elevated. How high the blood glucose level will rise before glucosuria occurs depends upon the renal threshold of each individual. In physiology, "renal threshold" is defined as that concentration of a substance in the blood plasma above which the substance is excreted by the kidneys and below which it is not excreted.

Although glucose is the sugar most commonly found in urine, other sugars such as lactose, fructose, galactose and pentose (rhamnose, arabinose, and xylose) may also be excreted.

627. Name some causes, other than diabetes, for glucose in the urine; list the reducing sugars and tell why they are so called; and cite the physiological cause of galactosuria, fructosuria, and pentosuria.

Conditions Associated with Glucosuria. You should know that diabetes is not the only cause of glucosuria. Kidney disease which affects the reabsorptive capacity for glucose of the renal tubules will also produce glucosuria. Pancreatic disease, endocrine disorders, and damage to the central nervous system may cause glucose excretion in urine. It may also be found during stress situations and pregnancy or associated with anesthesia. Another sugar, lactose, is detected more often in pregnant women, and it increases after delivery during lactation. Lactose is important because of its positive reaction in nonspecific tests for glucosuria.

Glucose, lactose, galactose, fructose, and pentoses (rhamnose, arabinose, and xylose) are all sugars which may be excreted in urine. They are called reducing sugars because of their chemical reaction in several nonspecific urine sugar tests, notably Benedict's reaction. This will be explained in more detail later in this section. The occurrence of glucosuria and lactosuria has been discussed. Galactosuria, fructosuria, and pentosuria are found in individuals with inherited metabolic defects. In addition, pentoses may be excreted after eating large amounts of fruits such as plums and cherries. Positive findings with the galactose and xylose tolerance tests (in which these sugars are ingested) will cause galactosuria and pentosuria. Reducing sugar tests on these urine specimens will be positive. Tests which are more specific (that is, glucose oxidase) are required to rule out the presence of glucose.

Exercises (627):

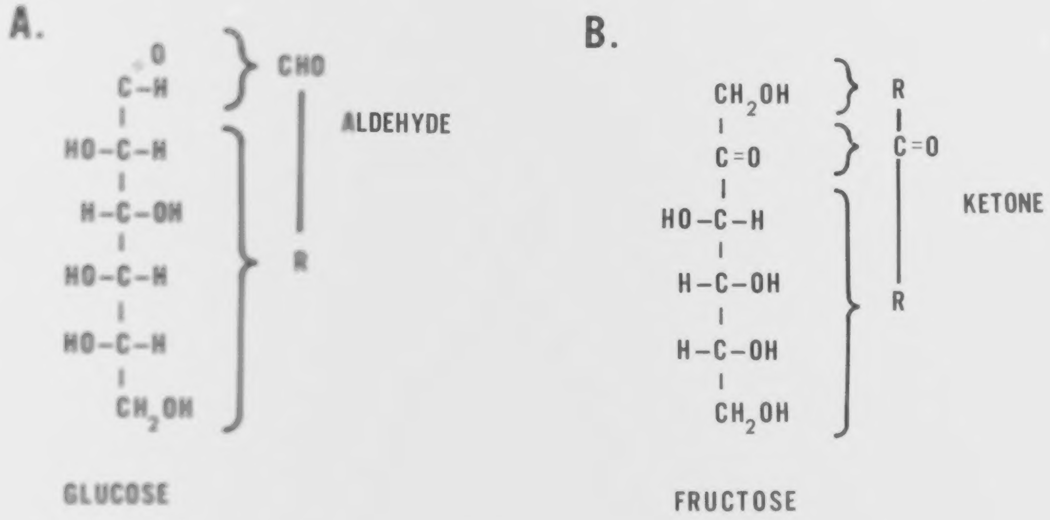
1. Give the causes for glucosuria, other than diabetes.

2. Name the reducing sugars and tell why they are given this name.

3. What type of disorder is responsible for galactosuria, fructosuria, and pentosuria?

628. Indicate whether given statements correctly reflect the reagents and principles used for detecting urine reducing substances and possible errors in testing.

Urine Reducing Substances. For years the detection of glucose by its chemical reducing effect has



A. Aldehyde with glucose.

B. Ketone with fructose.

Figure 5-1. Relationship between the general formulas for:

TABLE 5-3
POSSIBLE FALSE QUALITATIVE REACTIONS FOR GLUCOSE IN URINE

Urine Constituent	Benedict's Test	Reagent Test Tablet	Glucose Oxidase Test
Reducing Sugars Other Than Glucose			
Lactose	+	+	
Galactose	+	+	
Fructose	+	+	
Maltose	+	+	
Pentoses	+	+	
Drugs or Metabolites of:			
Ascorbic Acid (Vitamin C)	+	+	-
Antibiotics with Ascorbic Acid	+	+	-
Oxytetracycline			
Tetracycline			
Nalidixic Acid	+	+	
Cephalosporins	+	+	
Probenecid	+	+	
Chloral Hydrate	+		
Dipyrone			-
Dextrins (Pastry)	+	NR	
Glucosamine	+		
Indican	+	NR	
Isoniazid	+		
Meralluride			-
Para-amino-salicylic Acid	+		
Penicillin (Mass. Doses)	+		
Rhubarb	+		
Salicylates	+		
Santonin	+	NR	
Streptomycin	+		
Sulfonamides	+	NR	
Preservatives			
Chloroform	+	NR	
Formaldehyde	+	NR	
Cleaning Contaminants			
Hydrogen Peroxide			+
Sodium Hypochlorite (Bleach)			+
Menthol	+		
Phenol	+		
Turpentine	+		
Detergents			+
Other			
Creatinine	+		
Protein	+	NR	
Homogentisic Acid (Alkaptonuria)	+		
Uric Acid	+		

+ False Positive, - False Negative, NR No Report

been a routine procedure in urinalysis. Benedict's test is the classic method for this purpose. Benedict's qualitative reagent contains copper sulfate, sodium citrate, and sodium carbonate. In this alkaline solution, any compound that contains free aldehyde (R-CHO) or ketose (R-CO-R) groups will reduce the cupric ions (Cu^{++}) to cuprous ions (Cu^{+}) upon boiling.

In figure 5-1, you can see the relationship between the general formulas for aldehyde with glucose and ketone with fructose. A precipitate of cuprous oxide (Cu_20) is formed in the reaction. The color varies from green to yellow to red, depending upon the amount of reducing substance. The reaction with reducing sugars may be written as follows:

$$2\,Cu^{++} + \begin{array}{c}\text{Reducing}\\ \text{Sugar}\end{array} \xrightarrow[\text{Heat}]{\text{Alkali}} Cu_2{}^{+}O + \text{Oxidized Sugar}$$

Reagent Tablet. A commercial test tablet for reducing sugars is more often used than Benedict's reagent. It is a standard federal supply item (Urine

Sugar Test Tablet, NSN 6505-00-149-0220). The tablet contains copper sulfate and sodium carbonate as in Benedict's reagent. However, in addition, it contains sodium hydroxide and citric acid. The dry tablet is less stable chemically than the original liquid Benedict's reagent. Instructions are given with each bottle of tablets which detail observations associated with deterioration of the reagents. If these instructions are followed, the reagent tablet is considerably more advantageous than the liquid reagent. When a reagent tablet is added to urine and diluted with water, heat is generated by the sodium hydroxide as it dissolves and reacts with citric acid. The chemical principle is essentially that of the Benedict reaction. However, fewer false positive reactions due to reducing substances other than sugars are reported. Differences between the reagent tablet and the Benedict reaction compiled from the literature are indicated in table 5-3. Where differences have not been reported, we can assume that the reageant tablet reaction is the same as the Benedict reaction.

The approximate amount of reducing substance is indicated on the color chart. One precaution should be noted: the reaction must be observed as it occurs so that you will not miss a pass-through reaction. When the amount of reducing substance is greater than 2 percent, the reaction will rapidly "pass through" green, tan, and orange to a dark green-brown color. If this occurs, the result should be reported as over 2 percent without reference to the color chart.

You should become thoroughly familiar with the false reactions listed in table 5-3. Even though this list appears extensive, it should not be considered complete. Tetracycline and oxytetracycline antibiotics were first blamed for false positive reducing tests for glucose. Subsequently, it was discovered that ascorbic acid (vitamin C) added as a stabilizer to these antibiotics was the actual cause. Comparable levels of ascorbic acid are used with iron therapy in anemias, in vitamin supplements, and in processed foods to maintain an appetizing color. Up to 1 percent may be added to some candies to preserve their color. Although these sources of vitamin C are not mentioned specifically as the cause of false positive glucose (reducing) reactions, their ascorbic acid content should alert a wary technician. As you can see, table 5-3 may very well be expanded when more complete information is available.

Exercises (628):

Indicate whether each of the following statements is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. Benedict's test is a classic test for reducing sugars.

T F 2. The red chemical formed in a positive Benedict's reaction with glucose is cupric oxide.

T F 3. The dry tablet is more stable chemically than the original liquid Benedict's reagent.

T F 4. You must observe the reaction when using a urine sugar test tablet so that you will not miss a pass-through reaction.

T F 5. When the amount of reducing substance is greater than 5 percent, the reaction will rapidly "pass through" green, tan, and orange to a dark green-brown color.

T F 6. Ascorbic acid causes a false positive reaction with the reagent test tablet.

629. Identify the non-glucose reducing substances, conditions in which they are found, and methods of analysis.

Nonglucose Reducing Substances. The reagent test tablet reacts with all sugars. Lactose, galactose, fructose or pentose produce a positive reaction.

Lactose. Lactose is often excreted in the urine of lactating women. This condition normally ceases when lactation is completed. It may be found in trace amounts in the urine of 3- to 5-day old infants before their digestive systems have become fully developed, and in other children and adults who are deficient in intestinal lactose.

The reagent test tablet will detect the presence of lactose in urine. Identifying the sugar as lactose, though not a routine procedure, can be done by the mucic acid test, the osazone test, paper chromatography, and the galactose oxidase test. The presence of lactose in urine is usually considered physiological rather than pathological.

Galactose. Galactose is found in the urine of infants afflicted with galactosemia. These children are deficient in the enzyme necessary for converting galactose into glucose. This is a severe condition which can be corrected by eliminating lactose and other sources of galactose from the diet. If not done, the infants will rapidly deteriorate physically and mentally to an early demise. Occasionally, adults who ingest large quantities of milk or other lactose-containing foods will show trace amounts of galactose in the urine. This has no clinical significance and disappears upon the reduction of lactose intake. Galactose can be detected with the reagent test tablet and identified by the mucic acid test, osazone test, paper chromatography, and by the galactose oxidase test.

Fructose. Fructose sometimes occurs in the urine of patients with hepatic disorders. Its presence can be detected with the reagent test tablet. It can be identified by Selivanoff's test and by paper chromatography, neither of which is a routine procedure.

Pentose. Pentosuria is associated with certain types of drug therapy and with some hereditary conditions. In both cases, its presence in urine is considered benign, but can cause diagnostic problems. Its presence can be detected with CLINITEST*, and it will reduce Benedict's reagent at room temperature. Identification of a pentose can be done by Tauber's test, osazone test, and paper chromatography.

Exercises (629):

Match the following reducing sugars/conditions in column B by placing the letter beside the number of the column A item or items that most nearly describe it. Each element in column B may be used once, or more than once.

Column A	*Column B*
____ 1. Can be identified by the mucic acid test.	a. Lactose/lactosuria.
____ 2. Can be identified by Tauber's test.	b. Galactose/galactosuria.
____ 3. Occurs sometimes in urine of patients with hepatic disorders.	c. Fructose/fructosuria.
____ 4. Associated with certain types of drug therapy and some hereditary conditions.	d. Pentose/pentosuria.
____ 5. If not eliminated from diet, can cause rapid physical and mental deterioration in infants.	
____ 6. May be found in trace amounts in the urine of 3- to 5-day old infants before their digesitive systems have become fully developed.	

630. State the principle of reaction in the glucose oxidase method for urine glucose and cite the methods of interpretation.

Glucose Oxidase Method. The existence of so many false glucose reactions in copper reduction methods (Benedict's) left something to be desired for glucose screening. Modern advances in enzyme technology produced an apparent solution with the introduction of glucose oxidase. As you learned in your study of enzyme chemistry, an enzyme is specific in its reaction with a substrate. Sugars such as lactose, fructose, galactose, and pentose are not substrates for glucose oxidase and therefore do not react with this test. The general reaction of the enzyme glucose oxidase with glucose is as follows:

$$\beta\text{-d-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

From the earlier discussion of stereoisomerism and mutarotation of carbohydrates (Volume 2) you should realize that glucose has several chemical structures. This concept is of interest here because in the reaction given above the β (beta) form of d-glucose reacts, whereas the α (alpha) configuration is over 100 times *less* reactive. Anhydrous d-glucose (shelf reagent) is primarily in the latter form, while urine glucose consists of both forms in equilibrium.

An equilibrium between the alpha and beta forms of d-glucose occurs after about 30 minutes in aqueous solution. This information is useful when you wish to check the reaction of glucose oxidase reagent strips. For quality control a known solution of beta-d-glucose should be used to check this reaction periodically to insure valid results. A very reliable glucose solution readily available for this purpose is a freshly opened bottled of *Coca Cola.* Looking at the reaction again, you see that in the presence of glucose oxidase, glucose reacts with oxygen (O_2). This is atmospheric oxygen. It is provided by removing the reagent strip from the urine specimen *immediately* after dipping. Sufficient oxygen will not be available if the strip is left in the specimen.

Hydrogen peroxide (H_2O_2) produced in the previous reaction, oxidizes orthotolidine in the presence of peroxidase to form a blue color. This may be written:

$$H_2O_2 + \text{orthotolidine} \xrightarrow{\text{peroxidase}} \underset{\text{(blue color)}}{\text{oxidized orthotolidine}} + H_2O$$

Using glucose oxidase reagent test strip (CLINISTIX*) and the glucose test portions of the other strips containing the glucose oxidase test system, a color reaction is obtained specific for glucose. Tes-Tape, a reagent strip continuously impregnated with the test system, is based on the same principle and is available through federal supply sources.

Interpretation. With the chemistry and multireagent plastic strips, a negative reaction indicates no purple color and the test remains red. In a positive reaction, the test area is light to dark purple which corresponds to the amount of glucose present.

With the Tes-Tape a negative reaction is yellow, and positive reactions progress through several shades of green to a dark blue-green. The chemical reaction is the same glucose oxidase reaction, however.

The timing of the reactions is *critical* or *different* for each type of reagent strip. The multireagent, plastic strip must be read for glucose at 10 seconds after wetting. The continuous reagent strip (Tes-Tape) must be read first at 1 minute after wetting and 1 minute later (total 2 minutes) if the indication is 3+ or higher. The approximate concentration shown on the color chart should not be reported. The report should be 1+ or trace, and 2+ , 3+ , or 4+ as the color comparison indicated.

You may have assumed that false reactions are not possible in this test since glucose oxidase is an enzyme specific for glucose. If you will look again at table 5-3, you will see that both false negative and false positive reactions occur. False negative reactions involving ascorbic acid, vitamin C, are most important because of its extensive use. As little as 250 mg injected with an antibiotic is sufficient to inhibit the glucose oxidase reaction for glucose. Since the renal threshold for ascorbic acid is normally low, most of the vitamin is excreted in the urine. To complicate this difficulty even more, concentrations of vitamin C as low as 25 mg/% in urine react positively with the *Benedict test or reagent tablet.*

When vitamin C is present, you are faced with the dilemma of having *both* prime qualitative tests for glucosuria invalidated. Further, if you use a glucose oxidase reagent strip for initial screening, you will miss the reaction completely and not even be aware of it. The presence of ascorbic acid is not always so obvious as in its use as an antibiotic stabilizer. False negative reactions with the drugs dipyrone and meralluride, listed in table 5-3, are less important because of limited drug use. However, the fact that they have caused false negative tests for glucosuria prompts another question. What other substances now and in the future may cause false negative reactions with glucose oxidase?

The answer will be long in coming if the common practice of initial screening with glucose oxidase alone is continued. Benedict's, including the reagent test tablet, apparently has no known false negative reactions from interfering substances. In addition, some of the false glucose positive reactions may be clinically useful: that is, alkaptonuria, salicylates, and pentosuria. The detection of galactosuria, though very rare, may even save an infant's life. It seems apparent; therefore, that a need for the Benedict sugar test in urinalysis still exists. From the record to date, the Benedict reaction on a urine specimen should not be entirely discounted on the basis of an opposite result with the glucose oxidase test.

Exercises (630):

Complete each sentence with the appropriate word or words. A phrase rather than a single word may be required for the correct response.

1. The hydrogen peroxide (H_2O_2) produced in the reaction of glucose oxidase with glucose oxidizes ______ in the presence of peroxidase to form a ______ color.
2. Two reagents which may be used as known glucose specimens in the glucose oxidase screening test are ______ and __________ .
3. The multireagent plastic strip must be read at ______ after wetting.
4. The continuous Tes-Tape must be read first ______ after wetting and ______ later if indication is 3+ or higher.
5. The most serious false reaction in urine glucose screening with glucose oxidase and copper reduction methods is from __________ .
6. Concentrations of vitamin C encountered from therapeutic levels will cause false ______ glucose oxidase and false ______ copper reduction test for glucose.
7. If you use glucose oxidase as the only initial screening test, ______ will be entirely missed.
8. False negative reactions with glucose oxidase are obtained with the drugs ______ and __________ .

5-3. Miscellaneous Urine Tests

In this concluding section you will review a variety of tests. Except for the fact that they are all performed on urine, there is no clearly defined relationship among these various tests. Included in this discussion are laboratory procedures for urobilinogen, porphyrins, homogentistic acid and melanin, bilirubin, ketones, phenylketones, and occult blood. In addition, we will present a brief resumè of pregnancy testing. And finally you will review methods for setting up and maintaining quality control in urinalysis.

631. Explain the Ehrlich reaction, and specify the methods for testing, specimen preparation, sources of errors, and normal values for urobilinogen.

Urobilinogen. The subject of bile pigments and related pathology was introduced in Chapter 2, Volume 2 of this course. You will recall that urobilinogen is produced in the intestine by the action of bacteria on bile pigments. Part of this compound is absorbed into the blood and excreted in small amounts in the urine. Increased concentrations are found in diseases associated with excessive red cell breakdown as well as in other conditions.

The urine sample must be fresh and protected from daylight since urobilinogen will be converted to urobilin upon standing. Urobilinogen (as well as other bile pigments) is detected by means of the Ehrlich reaction in which urobilinogen reacts with paradimethyl-amino benzaldehyde in hydrochloric acid to form a red color. Bile pigments in the urine are first removed by absorption with calcium chloride or barium chloride. The procedure may be quantitated by the method of Wallace and Diamond in which Ehrlich's reagent is added to tubes containing serial dilutions of urine. The last tube showing a pink color is the endpoint.

Some investigators consider the Wallace and Diamond test outdated and prefer a modified Watson semiquantitative test. In the Watson method, Ehrlich's aldehyde reagent is reacted with urine after urobilin has been reduced back to urobilinogen with ascorbic acid. After formation of the aldehyde-urobilinogen complex, sodium acetate is added. The procedure is quantitated using the acid form of PSP (phenol red) dye. There appears to be no satisfactory quantitative procedure for urobilinogen that is uncomplicated and specific for urobilinogen. The addition of sodium acetate is advocated to enhance the color and, at least to some extent, inhibit color formation by indole and skatole (beta-methyl indole) derivatives.

A 1400- to 1600-hour specimen is usually used for this test. This is based on certain conclusions of Watson and coworkers that the output of urobilinogen is higher during this period. This is also a convenient time of the day to perform a test which must not be delayed. The urine must not be kept overnight. Perform the examination immediately, even though it is possible to reduce urobilin back to urobilinogen by the addition of ferrous hydroxide.

Urobilinogen is distinguished from porphobilinogen on the basis of chloroform solubility. Chloroform is added to the urine which has been reacted with Ehrlich's reagent. If the aqueous layer is red and the chloroform layer colorless, the test is positive for porphobilinogen. If the chloroform layer is red and the aqueous layer colorless, the test is positive for urobilinogen. If both layers are red, extract with chloroform until the chloroform layer is colorless. If the aqueous layer is still red, the test is positive for both porphobilinogen and urobilinogen.

Semiquantitative Reagent Strip Method. Urobilistix® and MULTISTIX® reagent strips are impregnated with para-dimethylaminobenzaldehyde and an acid buffer solution. They react with urinary urobilinogen, porphobilinogen and para-aminosalicylic acid to form colored compounds. The strip is dipped into fresh uncentrifuged urine collected without preservatives and removed free of excess urine. The color reaction is compared to the color shortly after 60 seconds.

There are five blocks provided on the chart. They range in color from light yellow to dark brown representing 0.1,1,4,8, and 12 Ehrlich units/100ml, which are within the normal range of values for urobilinogen. Accurate detection cannot be made on a decrease or absence of urobilinogen. No substances are known to clearly inhibit the reaction, but strongly alkaline urine will show higher urine urobilinogen values, and strongly acid urines will show lower urobilinogen levels.

Average Normal Values. Normal urine contains between 1 and 4 mg of urobilinogen in a 24 hour period. This is equivalent to less than 1 Ehrlich unit/24 hr collection period.

Exercises (631):

1. Explain the Ehrlichs reaction in the detection of urobilinogen.

2. How are the bile pigments removed from the urine in testing for urobilinogen?

3. In the Watson semiquantitative test for urobilinogen, why is the sodium acetate added?

4. Why must an analysis for urobilinogen be performed while the urine specimen is still fresh?

5. What time of the day is the two-hour specimen usually collected?

6. How would you distinguish between urobilinogen and porphobilinogen in terms of the Ehrlich reaction?

7. With what two reagents are the semiquantitative reagent strip for urobilinogen impregnated?

8. What substances are known to inhibit the reaction on the semiquantitative reagent strip for urobilinogen?

9. What are the average normal values for urobilinogen?

632. Indicate whether given statements correctly reflect the basic chemical characteristics of porphyrins, normal values, and methods of analysis.

Porphyrins. In our discussion thus far we have mentioned porphobilinogen. Actually, porphobilinogen is only one of a group of compounds classified as porphyrins or porphyrin precursors. Chemically, porphyrins are characterized by four pyrrole rings joined in a cyclic pattern by methene (CH) groups. Porphyrins form the basis of plant and animal respiratory pigments and involve very complex chemistry. In terms of clinical significance, porphyrins are usually divided into two major groups: (1) uroporphyrins and coproporphyrins which result from erythropoietic activity; and (2) porphobilinogen and 5-aminolevulinic acid (ALA) due to overproduction of these porphyrin precursors in the liver. Abnormal amounts of porphyrins are excreted in inborn errors of metabolism and acquired disorders of porphyrin metabolism. In inborn errors of metabolism, excessive production of porphyrins occurs in the liver. Acquired disorders of liver metabolism may result from alcoholic and nutritional liver cirrhosis, exposure to certain hepatotoxic chemical agents, and liver malignancies.

In lead poisoning, coproporphyrin III excretion in the urine is markedly elevated beyond the range that occurs in any other type of disease. Coproporphyrin excretion may rise from a norm of up to 250 μg/day to 40 times as high, at about 10 mg/day.

Coproporphyrin measurement is especially important for the diagnosis and daily management of patients with lead poisoning.

Normal values. Coproporphyrins are normally excreted in the urine in amounts ranging from 70 to 250 μg/day. Uroporphyrin excretion in the urine normally ranges from 10 to 30 μg/day. Delta-amino-levulenic acid excretion is between 1.0 and 7.0 mg/day.

Methods of determination. There are various procedures for the quantitative determination of urine porphyrins and their precursors. The best methods are quite involved and are usually performed only at Air Force reference laboratories. Simple methods, such as visual observation for color change of fluorescence under ultraviolet light, chemical procedure of boiling 50 ml of urine with 50 ml of 10 percent sulfuric acid, and the spectroscopic procedure requiring the addition of 1 ml of concentrated hydrochloric acid to 5 ml of urine, are all of limited usefulness. These procedures are outlined in AFM 160-49, *Clinical Laboratory Procedures—Chemistry and Urinalysis.*

Exercises (632):

Indicate whether each of the following sentences is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. Porphyrins are characterized by four pyrrole rings joined in a cyclic pattern by methene groups.

T F 2. The two main groups of porphyrins are (1) uroporphyrins and (2) coproporphyrins.

T F 3. Abnormal amounts of porphyrins are excreted in inborn errors of metabolism and acquired disorders of porphyrin metabolism.

T F 4. Coproporphyrin III excretion is markedly decreased in lead poisoning.

T F 5. Coproporphyrin measurement is especially important for diagnosis and daily management of patients with lead poisoning.

T F 6. Normal values for Delta-amino-levulenic acid excretion is 0.10 to 0.7 mg/day.

T F 7. The spectroscopic procedure for determination of porphyrins requires the use of ultraviolet light.

T F 8. The chemical procedure for porphyrin analysis requires boiling 50 ml of urine with 50 ml of 10 percent sulfuric acid.

T F 9. The simple methods for porphyrins are extensively useful in the diagnosis of diseases associated with abnormal porphyrins excretion.

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633. Indicate whether given statements correctly reflect the conditions which cause homogentisic acid and melanin in the urine and method of detection.

Homogentisic Acid and Melanin. Porphyrins are by no means the only pigments detectable in urine. Porphyrinuria, or the excretion of porphyrins in urine, is a relatively rare phenomenon. Although porphyrins impart an orange to red color to urine, abnormal coloration may be due to many other substances. An interesting and perhaps even more unusual clinical rarity is the brown to black color of *homogentisic acid* and the black color of *melanin (melanogen)*. Homogentisic acid in the urine results from the disease *alkaptonuria* which is an error of phenylalanine and tyrosine (amino acid) metabolism. Homogentisic acid is usually detected by the darkening of urine upon becoming alkaline, the ferric chloride test, or other screening procedures and is confirmed by paper chromatography. Melanin is a black pigment which is excreted in some cancer states (melanomas). The ferric chloride test is positive and confirmation of melanin is by means of chromatography. Darkening of urine also results from gentisic acid, indicans, and phenols.

Exercises (633):

Indicate whether each sentence is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. Homogentisic acid in urine results from the errors of phenylalanine and cystine metabolism.

T F 2. Patients with widespread melanotic tumors excrete melanogen in the urine.

T F 3. Homogentisic acid is detected and confirmed by the ferric chloride test.

T F 4. The ferric chloride test is positive for both melanin and homogentisic acid.

T F 5. Both melanin and homogentisic acid are confirmed by chromatography procedures.

634. Identify the methods used for testing urine bilirubin.

Urine Bilirubin. There are several tests that can be used to detect bilirubin in urine. However, most Air Force laboratories use a bilirubin test kit which employs a diazo reagent tablet. The test tablets (ICTOTEST) contain stabilized p-nitrobenzene p-toluene sulfonate, sulfosalicylic acid, and sodium bicarbonate. The kit comes complete with a mat consisting of asbestos and cellulose fibers. When a urine sample containing bilirubin is added to the mat dropwise, the bilirubin remains on the surface of the mat. You should always use the mats provided rather than filter paper or some other substitute because of this absorbent quality.

A test tablet is then placed on the mat and 2 drops of water are allowed to flow over the surface of the tablet. You should not add more than 2 drops of water. The purpose of the sulfosalicylic acid and sodium bicarbonate is to produce effervescence which will enhance the solubility of the tablet. If the mat shows a blue or purple color within 30 seconds, the test is considered positive. A slight pink color within 30 seconds is negative. The test is reported simply as positive or negative. The color of the tablet itself is not significant.

The test tablets just described react with both free (indirect or unconjugated) and direct (conjugated) bilirubin. False positive and false negatives are rare. Urobilin will not react, nor will salicylates. The sensitivity range is between 0.05 and 0.1 mg per 100 ml of urine. This meets the diagnostic requirements in most pathological processes. Urine which contains dyes such as Pyridium or Serenium may give a color reaction with bilirubin test tablets.

The *reagent strip test* is the simplest semiquantitative test for the determination of bilirubin. Reagent strips are impregnated with stabilized, diazotized 2, 4-dichloroaniline which reacts with bilirubin in urine to form a brown colored azobilirubin compound. The reagent strip is dipped into fresh, uncentrifuged urine, tapped to remove excess urine and, after a 20-second wait, compared to the color chart on the reagent strip bottle. The results are interpreted as negative or positive, with ranges of 1+, 2+, and 3+ (small, moderate, and large amounts of bilirubin). The sensitivity is the same as for the Ictotest tablet.

False positive reactions may occur with urine from patients taking large doses of chlorpromazine. Metabolites of certain drugs such as Pyridium or Serenium may give a reddish color. BILI-LABSTIX® and MULTISTIX® reagent strips also provide the same type of test area for determination of urinary bilirubin.

Exercises (634):

Match the following by placing the letter of the column B item beside the number of the column A item or items that most nearly describe it. Each element in column B may be used once, more than once, or not at all.

Column A

____ 1. False positive reactions may occur with urine from patients taking large doses of chlorpromazine.
____ 2. Pyridium may give reddish color reaction.
____ 3. Contains stabilized p-nitrobenzene p-toluene sulfonate, sulfosalicylic acid and sodium bicarbonate.
____ 4. Used to produce effervescence enhancing solubility.
____ 5. Sensitivity range is between 0.05 and 0.1 mg/100 ml of urine.
____ 6. Contains stabilized diazotized 2, 4-dichloroaniline.
____ 7. The reports can be reported as negative, or 1+, 2+, or 3+ positive.

Column B

a. Ictotest reagent tablet.
b. Bilirubin reagent strip test.
c. Sulfosalicylic acid and sodium carbonate.
d. Sulfosalicylic acid sodium hydroxide.

635. Indicate whether given statements correctly reflect the tests used for detecting ketones in urine, the specificity of the tests, basic reagent components, and possible sources of errors.

Ketonuria. The most commonly used tests for the detection of ketone bodies employ either *Ketostix®* or *Acetest®* tablets. Neither test detects β-hydroxybutyric acid, and *Ketostix®* strips are specific for acetoacetic acid. For practical purposes, there is no reason to distinguish among the ketone bodies. Hence, either of these two tests is adequate for clinical laboratory use.

In principle, the *Ketostix®* and *Acetest®* procedures are modifications of the Rothera test outlined in AFM 160-49, *Clinical Laboratory Procedures—Chemistry and Urinalysis.* That is, sodium nitroprusside in a suitable medium will react with certain ketones to produce a purple color. In the *Acetest®*, 1 drop of urine is placed on a tablet and the color is noted after a specified time interval. The reaction is read and reported as a small, moderate, or large amount. The sticks follow a similar pattern and are reported in the same way. The same reagents are now available on *Keto-Diastix®*, *Bili-Labstix®*, and *Multistix®* to measure ketones simultaneously with blood, glucose, protein, and pH and other constituents. A color chart on the bottle label has four color blocks, indicating negative, small, moderate, and large concentrations of ketones and ranging from buff to lavender and purple. False positive reactions may occur in patients receiving levodopa or phthalein compounds or when large concentrations of phenyl-ketones are excreted.

Exercises (635):

Indicate whether each of the following sentences are true (T) or false (F). If you indicate "false," explain your answer.

T F 1. Ketostix® and Acetest® tablets may adequately detect β-hydroxybutyric acid.

T F 2. Ketostix® strips are specific for acetoacetic acid.

T F 3. In principle, the Ketostix® and Acetest® are based on the reaction of sodium nitroprusside in a suitable medium to react with ketone to produce a purple color.

T F 4. The reaction for ketones is read as positive or negative.

T F 5. False positive reactions may occur in patients receiving levodopa or phthalein compounds.

T F 6. False negative reactions may occur when large concentrations of phenyl-ketones are excreted.

7. Which ketone bodies are detected by Ketostix®?

8. Which ketone bodies are detected by Acetest®?

636. Specify the method of testing for occult blood, name the reagent used, and cite a possible source of error.

Occult Blood. The term "occult" means obscure or hidden. By occult blood we mean blood that cannot be grossly observed. Test strips are available through Air Force supply channels which detect both free hemoglobin and the hemoglobin that is released from blood by chemical action of the test strip. Hemoglobin catalyzes the oxidation of orthotolidine by peroxide in the test strip, and a blue color results. Optimum pH for the reaction is provided by citrate buffers in the test strip. The reaction is observed at 1 minute and reported as small, moderate, or large amounts of blood according to the intensity of the blue color as illustrated in the Labstix indicator.

The test strips for blood should be stored in a cool area but not refrigerated. If the reagent test strip bottles contain a desiccant, it should be left in the bottle. It is important that strips be free of chemical contamination. Don't touch the test area of the strip or lay the strip on the workbench. In reading the color reaction, hold the strip close to the color chart under good lighting. Discolored test strips must not be used. The reagents described above are specific for hemoglobin and myoglobin, so false positives are rare. Ascorbic acid in the urine will inhibit the reaction and produce a false negative. Remember that antibiotics for injection contain large amounts of ascorbic acid which will produce urine levels high enough to interfere with this test.

Exercises (636):

1. State the principle of the hemoglobin test strip.

2. How is the optimum pH for reaction provided in the test strip?

3. Reagents in the test strips are specific for what substances?

4. What substances may cause false negative reactions?

637. Define phenylketonuria (PKU), state the technique for detecting urine PKU, and cite the reagents, principles, and method of interpretation.

Phenylketonuria (PKU). A disease known as *phenylketonuria,* or PKU, is an error of protein metabolism manifested by the presence of phenylpyruvic acid in the urine. It is caused by hereditary absence of the enzyme phenylalanine hydroxylase, essential for converting phenylalanine to tyrosine in its normal pathway of metabolism. Thus, the phenylalanine ingested in milk and other foods accumulates in the tissues and blood. By the age of four weeks and often much earlier, intermediate metabolites of phenylalanine, particularly phenylpyruvic acid, begin appearing in the infant's urine. When untreated, PKU results in brain damage and severe mental retardation. Although the incidence of this disorder is only one in 20,000 births, the consequence of PKU is so serious that early screening is imperative. However, when detected early and treated with a diet low in phenylalanine, the prognosis is good for normal mental development. Therefore, testing the urine of infants frequently during early life for the presence of phenylpyruvic acid is one of the most convenient ways of detecting phenylketonuria.

Reagent sticks are available for detecting phenylketones, principally phenylpyruvic acid. Reagent strips are impregnated with a solution containing ferric ions, specifically ferric ammonium sulfate, magnesium sulfate, and cyclohexylsulfamic acid, that form a specific bluish gray to gray-green color reaction with phenylpyruvic acid. The strips are buffered to prevent interference from phosphates. The strip is either dipped into a fresh sample of urine or pressed against a wet diaper. After exactly 30 seconds, it is compared to a color chart scaled at concentrations of 0.15 mg, 40 mg, and 100 mg phenylpyruvic acid per 100 ml urine. A false positive with the reagent strip frequently occurs. The reagent strips turn a pink to a purple color in urine containing salicylates and phenothiazine derivatives. Also, high concentrations of bilirubin or ammonia in the urine alter the normal color reaction developed with phenylpyruvic acid.

The reagent strips are inadequate for PKU testing of infants only a few hours old because they fail to show a positive urine test until several weeks after birth. Unless detected early, the infant suffers irreversible brain damage. Further, older infants with other symptoms of the disease do not always show a positive urine test even though the blood level of phenylketones is high.

Exercises (637):

1. What is phenylketonuria?

2. What convenient means of detecting PKU in urine is available?

3. With what reagents is the reagent strip impregnated for testing phenylpyruvic acid?

4. How do the color reactions obtained from salicylates and phenothiazine derivatives compare with those obtained for phenylpyruvic acid?

5. Why is the reagent strip inadequate for testing phenylketones in the urine of infants 24 hours after birth?

638. Indicate whether given statements correctly reflect the principle, the screening test, and quantitative methods used to detect phenylalanine in blood.

Blood Phenylalanine Screening Test. Many states now require the Guthrie test as the acceptable screening test for phenylketones. The Guthrie test is based upon the principle that growth of *Bacillus subtilis* is inhibited by beta-2-thienylalanine; but in the presence of phenylalanine, inhibition is overcome and growth takes place proportional to the concentration of phenylalanine. Zones of inhibition are read around paper discs impregnated with blood from a heel puncture. Control discs containing measured quantities of L-phenylalanine are used for comparison. Elevated phenylalanine plasma levels may be found before urine levels are increased.

The procedure for the Guthrie test has been modified and strictly standardized since its introduction. Earlier the Guthrie test had many inherent difficulties as a bioassay method, such as temperature control, culture purity, and standardization. A commercial kit is available which provides fairly specific, inexpensive, and realitively accurate concentrations below 20 mg phenylalanine/100 ml of blood. The blood specimen should be obtained 24 hours after the first milk feeding of the infant. As with the reagent strip, false positive results may be obtained and confirmation must be made with a quantitative method.

Quantitative Fluorometric Blood Phenylalanine Determination. Presently the only reliable quantitiative means of analysis is the fluorometric method. Careful measurement of plasma phenylalanine is a necessary laboratory function in following the dietary treatment of this disorder. In any case, the use of reagent impregnated strips or the ferric chloride test is inadequate in following dietary treatment. The test uses microsamples and is based on the fact that the fluorescence of the phenylalanine-ninhydrin-copper complex is greatly enhanced when formed in the presence of a peptide such as leucylalanine.

Exercises (638):

Indicate whether each of the following sentences is true (T) or false (F). If you indicate "false," explain your answer.

T F 1. The Guthrie test is based upon the principle that phenylalanine prevents the inhibition of a culture of *Bacillus anthracis* by beta-2-thienylalanine.

T F 2. In the Guthrie tests, zones of inhibition are read around paper discs impregnated with blood from a heel puncture.

T F 3. Elevated urine phenylalanine levels may be found before blood levels are increased.

T F 4. The blood specimen should be obtained 24 hours after the first milk feeding.

T F 5. As with the reagent strip, false positive results are obtained with the Guthrie test and require confirmation with the quantitative method.

T F 6. The quantitative fluorometric method is the method of choice for blood phenylalanine determination.

T F 7. Impregnated strips, the Guthrie tests, and the ferric chloride tests are all adequate in following dietary treatment.

T F 8. In the fluorometric method, leucylalanine, a peptide, causes an enhanced fluorescence of the phenylalanine-ninhydrin-copper complex.

639. Identify characteristics of human chorionic gonadotropin (HCG) and methods of detection by bioassay procedures.

Human Chorionic Gonadotropin (HCG). Most procedures for pregnancy tests measure HCG. HCG is a glycoprotein produced by trophoblastic cells beginning about 10 days after conception. The compound is formed by the union of two molecules, alpha and beta subunits, with a molecular weight of 28,000. The alpha units are considered nonspecific, since they are shared by the luteinizing hormone LH, the follicle stimulating hormone FSH, and the thyroid stimulating hormone TSH. The beta subunits are unique to HCG.

After conception a sharp rise in urinary HCG begins at 5 weeks after gestation, with the level increasing at about 10 weeks after the last menstrual period. As the level of progesterone and estrogen increases during the second trimester, HCG levels decline. For this reason, early pregnancy is confirmed by HCG which is the most significant measurement; however, for evaluation of fetal distress during the third trimester, estriol is the more meaningful.

Early Biologic Pregnancy Tests. At the present, bioassay tests for pregnancy—that is, tests utilizing experimental animals—must be considered largely of historical interest, but the technician should retain familiarity with the principles involved.

Aschheim-Zondek. The first reliable bioassay procedure for HCG was the Aschheim-Zondek or A-Z test. In the Aschheim-Zondek test, urine containing gonadotropic hormones produces marked changes in the ovaries of female mice. Four days following the first injection, the mice are sacrificed. In a positive test the ovaries are enlarged, hyperemic, and show hemorrhagic spots and yellow protrusions (corpora lutea). If the test is negative, the ovaries are small and white. The test is reliable, but the method is lengthy, and only specially trained technicians can effectively perform this test.

The Friedman test. In the Friedman test, urine containing gonadotropic hormones produces marked changes in the ovaries of mature female rabbits. Two injections of 10 ml each are given 24 hours apart. Forty-eight hours after the second injection, the rabbit is sacrificed and the ovaries are examined for the presence of hemorrhagic follicles which denote a positive test. Pale pink to white, unswollen ovaries are interpreted as a negative test. The rabbit or Friedman test is complicated and expensive, but when performed by experienced personnel it is very sensitive and at least 98 percent accurate. Menopausal urine has been reported to account for some of the false positive tests.

Utilizing amphibians. A number of tests utilizing amphibians have been developed. Two of these tests employ the male leopard frog, *Rana pipiens*, and female toad, *Xenopus laevis*. Male leopard frogs injected with urine or serum containing gonadotropic hormones void spermatozoa in their urine. The test requires 2 to 3 hours to read, and negative frogs should be injected with gonadotropin as a control measure. The test is reported to be 95 percent accurate, although some seasonal variation is experienced. *Rana pipiens* are used except during May and June, when frogs of the species *Rana clamitans* are used.

Female African toads (*Xenopus laevis*) ovulate when injected with urine containing gonadotropic hormones. The toad is placed in a glass jar on wide-mesh, wire screen flooring suspending 1 inch from the bottom. Water is added to a depth of 3 inches. The toad sits on the screen and the gelatinous ova pass through the screen. These ova can be seen macroscopically and indicate a positive test. The test is reported negative if no ova are extruded after 24 hours. The African toad is quite difficult to procure and maintain, and a number of drugs interfere with this test.

Drugs excreted in the urine may decrease sensitivity and produce false negative results or even death of the test animal. Some drugs causing interference are quinidine, barbiturates, laxatives, antihistamines, sulfonamides, salicylates, antibiotics, ergot, and morphine derivatives.

Exercises (639):

Identify the hormone, hormonal unit, or test in column B with the information to which it is closely related in column A by placing the letter of the column B item beside the appropriate column A item. Each column B item may be used once, more than once, or not at all.

Column A

____ 1. Level increases during the second trimester of pregnancy.

____ 2. Level decreases during the second trimester of pregnancy.

____ 3. Most significant measurement to confirm early pregnancy.

____ 4. A more meaningful measurement of fetal distress during the third trimester.

____ 5. A glycoprotein produced by trophoblastic cells beginning 10 days after conception.

____ 6. Shared by LH, FSH, and TSH.

____ 7. Unique to HCG.

____ 8. The first reliable bioassay procedure using urine containing HCG injected into female mice that produce marked changes in their ovaries.

Column B

a. HCG.
b. Alpha and beta subunits.
c. Alpha subunits.
d. Beta subunits.
e. Progesterone and estrogen.
f. Aschheim-Zondek test.
g. Friedman test.
h. *Rana pipiens*.
i. *Xenopus laevis*.
j. Estriol.

Column A

___ 9. Male leopard frogs injected with serum or urine containing HCG void spermatozoa in their urine.

___ 10. Urine containing HCG injected into mature female rabbits; forty-eight hours after, ovaries are examined for presence of hemorrhagic follicles.

___ 11. Female African toad that ovulates when injected with urine containing HCG.

640. Indicate whether given statements correctly reflect the principles of immunologic slide tests for pregnancy, influencing factors, and possible sources of errors.

Immunologic Pregnancy Tests. Immunologic pregnancy tests based on detection of HCG have largely replaced bioassays for routine clinical use. Although bioassay can give reliable results, they require more time and attention for proper calibration and control than immunologic methods. Nevertheless, despite their simplicity and speed, HCG immunoassays definitely require careful standardization. The majority of the techniques available are based upon an agglutination-inhibition reaction utilizing either red cells or latex particles.

Immunologic tests for HCG are by far the most practical and widely used for the qualitative determination of human chorionic gonadotropin. Since HCG is antigenic, it has been possible to prepare an antiserum. Several immunologic methods have been developed to detect chorionic gonadotropin utilizing complement-fixation, agglutination-inhibition, and precipitin reactions.

Slide tests. Most of the presently available slide tests from Wampole, Ortho, Hyland, Organon, Roche, Burroughs, Wellcome, and Lederle are based on latex particles agglutination inhibition. Latex particles coated with human chorionic gonadotropin serve as the antigen. One drop of the patient's urine is placed on a slide and mixed with a drop of chorionic gonadotropin antiserum. If the urine is from a nonpregnant individual (no gonadotropin present), the unchanged antiserum subsequently reacts with the coated latex particles to produce macroscopic agglutination. If the patient is pregnant, the urine chorionic gonadotropin neutralizes the antiserum so that it is no longer free to react with the latex particles to bring about clumping. Positive and negative reactions are shown in figure 5-2. The 2-minute latex inhibition slide test for pregnancy is often reserved for STAT cases when ectopic pregnancy or incomplete abortion cases are suspected.

Influencing factors. Pregnancy test reagents must not be frozen, but should be maintained at refrigerator temperature when not in use. All reagents should be brought to room temperature before use. Any urine specimen, first in the morning or random, may be used. Specimens with a specific gravity of at least 1.015 are preferred for greater accuracy. Further, specimens should be tested within 12 hours of collection unless they are adequately preserved. Do not attempt to test specimens which are grossly contaminated or which contain blood or abnormally high protein levels. The slide should be rocked gently, not vigorously. Improper mixing of the specimen and the reagents may lead to erroneous results. The mixture must not be allowed to dry on the slide. The slide should be examined no longer than 2 minutes following addition of the antigen to the urine-antiserum mixture. Slides should not be placed on a warm surface such as the view box used in Rh testing. Positive urine may be used as a positive control. Samples of positive urine may be kept frozen in aliquots for convenient thawing and use. HCG in urine is stable for 4 months in the freezer (18°C), but at room temperature it is stable for only 72 hours. You must note the fact that the accuracy of such immunologic tests is influenced by so many factors that authorities expect erroneous results in about 5 to 6 percent of the tests. Much consideration should be given to performing the 2-hour test, since it may be stated that the 2-hour tube tests are more sensitive than the slide test.

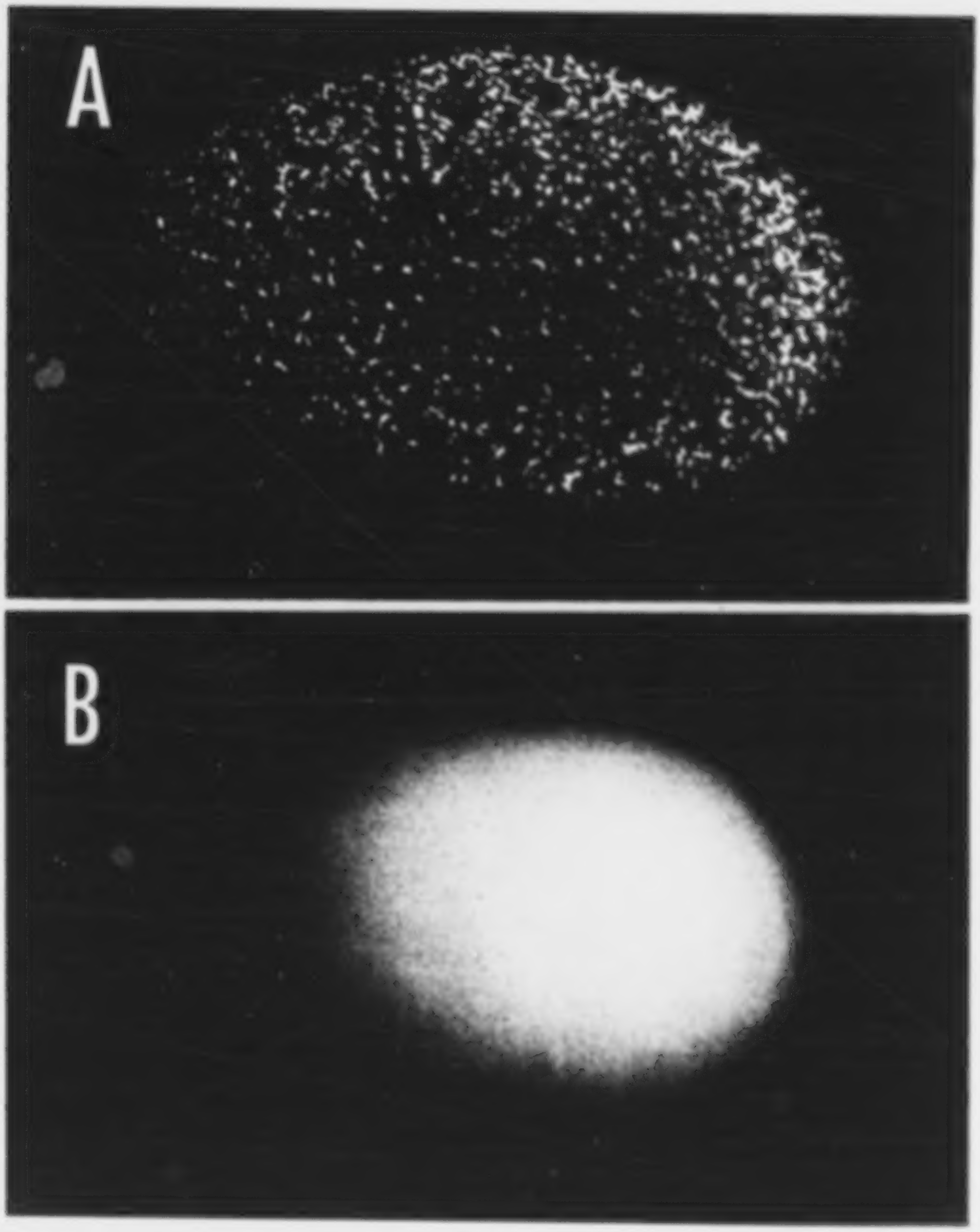
Exercises (640):

Indicate whether the following sentences are true (T) or false (F). If you indicate "false," explain your answer.

T F 1. Immunologic tests for pregnancy have largely replaced bioassay tests, since they require less time and attention for calibration and control.

T F 2. The majority of the technique used for immunologic tests are based upon an agglutination-inhibition reaction utilizing WBCs and latex particles.

T F 3. Most slide tests are based upon HCG utilizing complement-fixation.



A. Negative test.

B. Positive test.

Figure 5-2. Positive and negative reaction to the two minute latex inhibition slide test.

T F 4. Latex particles coated with human chorionic gonadotropin serve as the antigen.

T F 5. If the urine is from a nonpregnant individual, the unchanged antiserum reacts with the coated particles to produce a macroscopic reaction (negative test).

T F 6. Since the 2-minute slide test is more sensitive than the 2-hour test, it should be routinely used in the laboratory.

T F 7. Pregnancy test reagents must not be frozen but maintained at refrigerator temperature.

T F 8. Grossly contaminated or specimens containing blood or abnormally high protein levels may be used for slide pregnancy tests.

T F 9. HCG in urine is stable for 4 months in the freezer, but at room temperature it is stable for 72 hours.

T F 10. Erroneous results may be expected in about 5 to 6 percent of the slide tests, according to authorities.

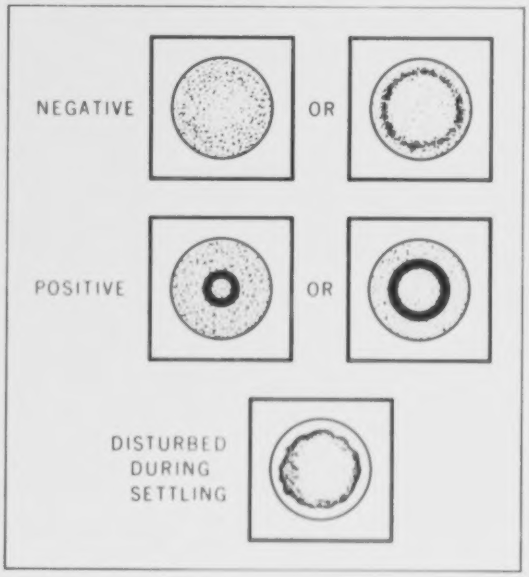
641. State the principle of the 2-hour tube test, and cite methods, interpretation, and clinical applications for pregnancy tests.

Two-Hour Pregnancy Tests. The two-hour tests for pregnancy presently available from Wampole, Organon, Ames Burroughs Wellcome, and Roche are based on the agglutination-hemagglutination, inhibition reaction. An antigen-antibody system is used. Red blood cells or latex particles coated with HCG are used as antigens. In the presence of anti-HCG antiserum, the HCG-coated cells or latex particles agglutinate and settle out. The markers of the antigen antibody reaction are the red cells and the latex particles. When HCG in the urine is added to the anti-HCG antiserum prior to the addition of the HCG-coated red cells or latex particles, the HCG present in the urine neutralizes the antiserum so that it fails to agglutinate the HCG coated cells or latex particles that are subsequently added. Absence of agglutination thus indicates a positive pregnancy test. When agglutinated erythrocytes in low concentration settle in a test tube with a hemispheric bottom, they form a uniform film which covers the bottom of the tube, as shown in the negative examples of figure 5-3. This indicates a negative pregnancy test. However, when unagglutinated erythrocytes settle in such a tube, they form a sharply demarcated ring or "doughnut," as shown in the positive examples of figure 5-3. This indicates a positive pregnancy test. With gradually increasing agglutination, the ring increases in size, becomes very faint, and disappears. You should remember that if the antiserum has not been neutralized, the RBCs will be agglutinated, and a diffuse mat of cells (with or without a faint ring) will form in the bottom of the tube, as noted in the negative examples of figure 5-3.

The UCG and the Prognosticon accuspheres show the same degree of reliability and sensitivity. Authorities feel that they can be considered reliable even for the early diagnosis of pregnancy. In addition, both tests make it possible to introduce quality control in pregnancy testing. This is a factor which had been ignored by many laboratories performing pregnancy tests. Manufacturers of the UCG test have claimed that with their procedure pregnancy can be determined as early as 4 days after the date of the missed period. Studies with the Pregnosticon test have shown that the test is accurate starting with the eighth day after the missed period. A Federal stock listed item is available for 2 hour pregnancy tests: Test Kit, pregnancy determination, 25 tests - (6505-00-080-0617).

Serum may be used and processed according to manufacturer's instructions. The reagents must be added in the correct order. For most tests all reagents must be at room temperature and should be well mixed. The tube test may be considered more sensitive than the slide test.

Clinical Applications. The quantitative HCG



Negative: a diffuse mat of red cells.

Positive: a clear cell ring at the bottom of the test tube similar to control.

Figure 5-3. Basic interpretation of readings for 2-hour pregnancy test.

assay may be useful as a guide to prognosis of threatened abortion during the first trimester. It has been further valuable in the diagnosis of hydatidiform mole. Choriocarcinoma is a malignancy of trophoblastic tissue which women usually develop from hydatidiform mole. In males choriocarcinoma is an unusual but highly malignant testicular tumor.

Pregnancy Test by Radioimmunoassay. More recently, radioimmunoassay tests have become available. The beta subunit radioimmunoassay for HCG is by far the most sensitive and specific method available. Presently, the beta subunit HCG radioimmunoassay is not normally used routinely as a pregnancy test, but reserved for study of patients being treated for choriocarcinoma or hydatidiform mole.

Exercises (641):

1. In the 2-hour tube test for pregnancy, what is the antigen used in the antigen-antibody reaction? The antibody?

2. What happens to the HCG antiserum when a urine specimen containing HCG is added?

3. When the absence of agglutination denotes a positive pregnancy test, what distinctive appearance is noted at the bottom of the test tube?

4. What significant factor does the 2-hour pregnancy test introduce in pregnancy tests?

5. What are some of the reasons for quantitative HCG assay?

6. What specific test, considered highly specific and sensitive, is reserved for study of patients being treated for choriocarcinoma or hydatidiform mole?

642. State some essential requirements for quality control in urinalysis.

Initiating Quality Control. First, consideration should be given to maintaining an up-to-date book of standard operating *approved* procedures. Since urinalysis is often considered the "stepchild" of the clinical laboratory, transient technicians tend to use their own modifications of tests. It is important that all reagents and references correspond to those in the procedures book. Dipsticks, bottles, and other chemicals should be labeled with the dates of initial opening and use. Dates of expiration of test kits must be followed even though they appear to "work" well.

Handling specimens. Many errors in urinalysis stem from the initial collecting and handling of specimens. It is most desirable that an early-morning midstream sample be obtained and examined within 30 minutes. Prior to testing—that is, before you pour that sample into the centrifuge tube—mix the urine well. In addition, problems associated with specimen handling, which include letting the specimens stand for hours and giving the patient too much water to drink, must be controlled.

The use of reference samples assures that reagents, reagent strips, and instrumentation are in working order and also monitors the technique of the individual performing the procedures.

Reference controls available. Commercial sample controls should be obtained through your local purchase supply channels. Commercial urine control samples include Ames "Tek-Chek" which is supplied in freeze-dried form. It contains a combination of positive and negative constituents for protein, glucose ketones, glucose, bilirubin, and blood. Specific gravity and pH value may be evaluated on all of these control specimens. Harleco's "Unitrol," Hyland's "Q-Pak," Chemistry Urine Control Supplement and Lederle's controls are available for qualitative and quantitative urinalysis procedures.

To get the most meaningful feedback, it is recommended that these controls be tested in the same manner as the patient samples. The control specimen may be "hidden" among the routine samples in such a way that the technician performing the test does not know which specimen is the control.

Proficiency Testing. Urinalysis can be monitored by both internal daily checks, and external proficiency survey testing measures. Proficiency testing provides an overview of the individual laboratory's quality control program. Each participant is given an opportunity to observe its own standing in relation to either peers or reference laboratories.

It has become apparent that some methods are not particularly good for certain determinations and presumably should be abandoned. The technician must make every effort to keep abreast of approved standard techniques in urinalysis. Practice of current approved techniques complements accuracy and reliability for good quality control in urinalysis.

Exercises (642):

1. What should be a first consideration in initiating quality control in urinalysis?

2. What type of urine specimen is most desirable? How soon should it be examined?

3. Before pouring a urine sample into the centrifuge tube, what should first be done with the urine?

4. What purpose does the use of reference samples serve?

5. List some commercial reference controls.

6. How should these controls be tested for the most meaningful information?

7. In what two ways can urinalysis be monitored by proficiency testing?

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Note: None of the items listed in the bibliography above are available through ECI. If you cannot borrow them from local sources, such as your base library or local library, you may request one item at a time on a loan basis from the AU Library, Maxwell AFB, Alabama, ATTN: ECI Bibliographic Assistant. However, the AU Library generally lends only *books* and a limited number of *AFMs*. TOs, classified publications, and other types of publications are *not* available.

Answers for Exercises

CHAPTER 1

Reference:

600 - 1. T.
600 - 2. T.
600 - 3. F. Use the fine focus adjustment.
600 - 4. T.
600 - 5. F. It is never used with an artificial light source.
600 - 6. F. The filament focus control will move the lamp tube back and forth.
600 - 7. F. A more accurate method is to take a second flat mirror and place it so that you can see the underside of the condenser iris diaphragm.

601 - 1. F. Both the condenser iris diaphragm and the lamp iris diaphragm should be closed.
601 - 2. T.
601 - 3. T.
601 - 4. F. The condenser iris diaphragm is intended to serve as a part of the optical system, which is responsible for resolution of the image.

602 - 1. Remove one of the oculars.
602 - 2. Close the iris until approximately two-thirds to three-quarters of the diameter of the back lens is fully illuminated.
602 - 3. To produce the desired reduction in illumination of the back lens of the objective.
602 - 4. The intensity of illumination is controlled with neutral density filters. Some lamps may be equipped with a rheostat which may also be used for this purpose.
602 - 5. Use lint-free tissue or a soft cloth.

603 - 1. c.
603 - 2. d.
603 - 3. d.
603 - 4. a.
603 - 5. c.
603 - 6. F. They are of value only when made on properly collected and prepared 24-hour specimens.
603 - 7. F. For certain types of urine analyses, they must be kept in the refrigerator. If the outside of the bottle is clean and free from urine, the other contents of the refrigerator will not be contaminated.
603 - 8. T.
603 - 9. F. Usually it has no relationship to the time the patient voided.
603 - 10. T.
603 - 11. F. Unless used carefully, the antiseptic might render the urine sterile before it is cultured.
603 - 12. F. It should at least be washed with detergent and hot water.
603 - 13. F. It is not a requirement, but urine bottles from a contagious case must certainly be sterilized.
603 - 14. F. Ordinary detergents do *not* inactivate viruses or even eradicate bacteria.
603 - 15. T.

604 - 1. T.
604 - 2. T.
604 - 3. F. Toluene extracts free steriods, and unless the specimen is mixed thoroughly, part of the free steroids will be lost and low values will be obtained.
604 - 4. T.
604 - 5. F. Thymol gives a false positive for albumin and may interfere with tests for bile.
604 - 6. F. A strong mineral acid must be used; for example, concentrated HCl to pH3.

CHAPTER 2

605 - 1. Glomerulus, proximal convoluted tubule, loop of Henle, and distal convoluted tubule.
605 - 2. e.
605 - 3. g.
605 - 4. c.
605 - 5. h.
605 - 6. b.
605 - 7. i.
605 - 8. f.
605 - 9. a.
605 - 10. d.
605 - 11. A. Afferent arteriole.
B. Efferent arteriole.
C. Glomerulus.
D. Bowman's capsule.
E. Proximal convoluted tubule.
F. Loop of Henle.
G. Distal convoluted tubule.
H. Collecting tubule.

606 - 1. Remove nitrogenous wastes, regulate blood volume, control water content of tissue, and maintain pH and chemical composition of body fluids.
606 - 2. With filtration of plasma-like fluid through the glomerular capillaries.
606 - 3. The blood pressure in Bowman's capsule is about twice that of any other capillary in the body.
606 - 4. In the absence of compensating factors, a rise in blood pressure increases the rate of glomerular filtration and hence increases urine production. A decrease in blood pressure has the opposite effect.
606 - 5. Stimulation of the splanchnic (visceral) nerve decreases renal blood flow and therefore the rate of filtration and vasodilators such as adrenalin also decrease glomerular filtration after an initial phase of vasoconstriction.

606 - 6. Proteins (which include albumin) are usually unable to pass through the walls of the glomerular capillaries, which act as filters, because the proteins have a molecular weight of more than 68,000.
606 - 7. In the proximal convoluted tubules.
606 - 8. This substance appears in the urine.
606 - 9. The antidiuretic hormone (ADH), which is produced by the pituitary gland.
606 - 10. Sodium, bicarbonate, chloride, amino acids, and phosphates.
606 - 11. Creatinine, ammonia, potassium, and foreign substances, like penicillin and dyes.
606 - 12. 100 to 150 liters of glomerular filtrate to 1 to 1.5 liters of urine, or 100:1.
606 - 13. (1) Ketone bodies are oxidized to corresponding organic acids; (2) ammonium ions replace sodium ions; (3) bicarbonate ions are reabsorbed in the tubules; (4) hydrogen ions are excreted by the tubules.
606 - 14. Glomerular filtrate: pH 7.4; specific gravity 1.008 to 1.012. Urine: pH 4.6 to 8.0; specific gravity 1.015 to 1.025.

607 - 1. h.
607 - 2. i.
607 - 3. a.
607 - 4. d.
607 - 5. b.
607 - 6. c.
607 - 7. f, g.
607 - 8. e.

CHAPTER 3

608 - 1. i.
608 - 2. l.
608 - 3. e.
608 - 4. b.
608 - 5. c.
608 - 6. g.
608 - 7. h.
608 - 8. j.
608 - 9. f.
608 - 10. k.
608 - 11. d.
608 - 12. a.

609 - 1. (a) Pigments which arise from bile metabolism; (b) certain vegetable pigments from diet, such as the pigment in beets and carrots; (c) certain drugs or their metabolites, such as methylene blue dye and various sulfa drugs.
609 - 2. They may confuse color comparisons made in urine strip screening tests.
609 - 3. b, d.
609 - 4. c.
609 - 5. a.
609 - 6. e.
609 - 7. f, h.
609 - 8. i.
609 - 9. g.
609 - 10. Mixing with activated charcoal and filtering.
609 - 11. No assumptions can be made solely on the basis of urine color.
609 - 12. b.
609 - 13. d.
609 - 14. a.
609 - 15. c.

610 - 1. The reaction changes to alkaline because of the formation of ammonia from urea.
610 - 2. Strongly acid urine.
610 - 3. Strongly alkaline.
610 - 4. To control urinary calculi or to provide an effective pH range for antibiotic therapy.

611 - 1. Methyl red and bromthymol blue; pH 5 to 9.
611 - 2. The increments on the color scale are too great for close pH approximations.
611 - 3. Acid or alkaline fumes, previously wet strips, and leaching reagent by soaking or excess wetting.
611 - 4. The desiccant is there to absorb moisture from the atmosphere which enters the bottle when it is open. Unless the reagent strips are kept dry, they will not remain stable.

612 - 1. T.
612 - 2. F. 0.001 is added to the sp gr reading obtained for each 3°C above or below the calibration temperature.
612 - 3. F. The three digits after the decimal point would be multiplied by 3.
612 - 4. F. The reading would be 1.012.
612 - 5. F. All new urinometers should be checked for accuracy.

613 - 1. The refractive index is the ratio of the velocity of light in air to the velocity of light in a solution.
613 - 2. Dissolved solids.
613 - 3. In the measurement of small quantities of urine.
613 - 4. To the nearest 0.001 unit.
613 - 5. (a) Corrected temperature value read directly from internal scale; (b) calibration easily checked against refractive index of water; (c) time saved in taking multiple specific gravity readings; (d) freedom from tedious manipulations.

614 - 1. F. The osmolality of urine is an indicator of the amount of osmotic work done by the kidneys.
614 - 2. T.
614 - 3. F. Osmolality depends only upon the number of particles of solute in a unit of solution.
614 - 4. F. Measurement of osmolality of a solution is a measure of the number of osmols in one kilogram of solution.
614 - 5. T.
614 - 6. F. The osmolal clearance reflects the ability of the kidney to conserve or excrete water.
614 - 7. T.
614 - 8. T.
614 - 9. T.
614 - 10. T.
614 - 11. F. The differences between the freezing points and vapor pressures of water and of an aqueous solution are directly proportional to the molality of the solutions.
614 - 12. T.

CHAPTER 4

615 - 1. By "organized sediment" we mean body cells and their derivatives which are found in urine. The definition could be expanded to include other cellular elements such as yeast, parasites and bacteria; however, this is not the usual understanding.
615 - 2. Blood cells, epithelial cells, spermatozoa, and casts.
615 - 3. Starch granules turn blue upon the addition of iodine.
615 - 4. Yeast cells are usually identified by the presence of budding.

616 - 1. There is a chance that more significant sediment will be observed after centrifugation.
616 - 2. No more than two specimens.
616 - 3. Use 10 ml. All elements except casts should be reported in number per high power field.

616 - 4. The amount should be minimal, not critical, because if too much acetic acid is added, red blood cells and casts will be destroyed.
616 - 5. To clear amorphous urates, add an equal part of warm physiological saline to the urine before centrifuging.
616 - 6. Three parts of solution A and 97 parts of solution B.
616 - 7. Solution A. Methylrosaniline chloride (crystal violet). Solution B. Safranine.

617 - 1. F. It is now believed that it is difficult, if not impossible, to state where in the urinary track epithelial cells originate.
617 - 2. F. They do not always come from the kidney.
617 - 3. T.
617 - 4. T.
617 - 5. F. They are referred to as *pus* cells.
617 - 6. F. Unless accompanied by five or six red blood cells per hpf, will not give a positive benzidine test.
617 - 7. T.
617 - 8. Pyuria.
617 - 9. Renal parenchyma.
617 - 10. Oval fat bodies.
617 - 11. Glitter cells.

618 - 1. The presence or appearance of blood in the urine, gross or only a few cells.
618 - 2. Carcinoma, chronic inflammation, renal calculi, nephritis, cystitis, hemorrhagic disease, trauma to kidneys.
618 - 3. pH, specific gravity, age.
618 - 4. Add acetic acid and if the cells disappear they were RBCs; second, use Hemastix, or the hemoglobin area of Bili-Labstix, or Occultest reagent tablets.
618 - 5. Hypertonic.
618 - 6. Ghost forms.

619 - 1. g.
619 - 2. g.
619 - 3. h.
619 - 4. i.
619 - 5. j.
619 - 6. k.
619 - 7. k.
619 - 8. l.
619 - 9. a.
619 - 10. b.
619 - 11. b.
619 - 12. c.
619 - 13. d.
619 - 14. e.
619 - 15. f.

620 - 1. T.
620 - 2. F. They are soluble in acetic acid.
620 - 3. F. They are colorless, highly refractile prisms, varying in size and presenting 3, 4, or 6 sides, giving the typical coffin-lid forms.
620 - 4. T.
620 - 5. T.
620 - 6. T.
620 - 7. F. They are soluble in alkali and upon warming.
620 - 8. F. They may also appear in neutral and alkaline urine.
620 - 9. T.
620 - 10. T.

621 - 1. e.
621 - 2. a.
621 - 3. b.
621 - 4. b.
621 - 5. c.
621 - 6. a.
621 - 7. d.
621 - 8. f, g.

CHAPTER 5

622 - 1. Plasma proteins.
622 - 2. Nonserum proteins in urine are mucoproteins.
622 - 3. Temporary impairment of renal circulation which results from postural aberration—showing protein in the urine.
622 - 4. (a) Extraordinary physical exercise, (b) ingestion of high protein meal, (c) postural aberration, (d) bleeding in urogenital tract, (e) febrile conditions.
622 - 5. (a) The clinical importance of the results; (b) the limitations of any single method.
622 - 6. No qualitative urine protein screening test *measures* a specific protein fraction; the fraction is only identified.
622 - 7. (a) Colorimetric reagent strip test (and indicator with buffer) (b) sulfosalicylic acid, (c) heat and acetic acid.

623 - 1. (a) 24-hour urine volume, (b) diet, (c) urinalysis method, (d) normal physiological fluctuations.
623 - 2. 7 to 10 mg/100 ml of urine.
623 - 3. If the screening test is so sensitive that it detects less than the higher limits or normal (7 to 10 mg/100 ml), positive results will be obtained on normal amounts of urine protein. This would render the test useless for screening urine specimens.
623 - 4. Because of its nonspecific reactions with many other normal urine constituents.

624 - 1. c, d.
624 - 2. a.
624 - 3. b.
624 - 4. e.
624 - 5. c.
624 - 6. g.
624 - 7. h.

625 - 1. T.
625 - 2. F. Positive reaction for protein.
625 - 3. T.
625 - 4. T.
625 - 5. F. They do not cause false positive reaction with the buffered acid reagent.

626 - 1. Protein.
626 - 2. Acid, pH3.
626 - 3. 5, 20.
626 - 4. 30.
626 - 5. pH, protein.
626 - 6. Albumin.

627 - 1. (a) Kidney disease, (b) pancreatic disease, (c) endocrine disorders, (d) damage to central nervous system, (e) stress, (f) pregnancy, (g) anesthesia.
627 - 2. Glucose, lactose, galactose, fructose, and pentoses. Because of their chemical reaction in several nonspecific urine sugar tests.
627 - 3. Inherited metabolic defects.

628 - 1. T.
628 - 2. F. Cuprous oxide.
628 - 3. F. The dry tablet is less stable.
628 - 4. T.
628 - 5. T.
628 - 6. T.

629 - 1. a, b.
629 - 2. d.
629 - 3. c.
629 - 4. d.
629 - 5. b.
629 - 6. a.

630 - 1. Orthotolidine; blue.

630 - 2. Equilibrated glucose solution from shelf reagent: Coca Cola.
630 - 3. 10 seconds.
630 - 4. 1 minute; 1 minute.
630 - 5. Vitamin C (ascorbic acid).
630 - 6. Negative; positive.
630 - 7. Positive glucosuria.
630 - 8. Dipyrone; meralluride.

631 - 1. In the Ehrlich reaction, urobilinogen reacts with paradimethyl-amino benzaldehyde in hydrochloric acid to form a red color.
631 - 2. By asborption with calcium chloride or barium chloride.
631 - 3. To enhance the color and to some extent inhibit color formation by indole and shatole derivatives.
631 - 4. Urobilinogen will disappear from urine as it is readily oxidized to urobilin.
631 - 5. 1400 to 1600 hr.
631 - 6. Urobilinogen can be extracted with chloroform; the red porphobilinogen complex is not soluble in chloroform.
631 - 7. Paradimethylaminobenzaldehyde and an acid buffer solution.
631 - 8. None, as yet.
631 - 9. Between 1 and 4 mg/24 hr, less than 1 Ehrlich unit/24 hr collection period.

632 - 1. T.
632 - 2. F. (1) Uroporphyrins and coproporphyrins and (2) porphobilinogen 5-aminolevulinic acid (ALA).
632 - 3. T.
632 - 4. F. It is markedly elevated.
632 - 5. T.
632 - 6. F. It is 1.0 to 7.0 mg/day.
632 - 7. F. The procedure requires the addition of 1 ml of concentrated hydrochloric acid to 5 ml of urine.
632 - 8. T.
632 - 9. F. Simple methods are all of limited usefulness.

633 - 1. F. It results from the disease *alkaptonuria*, which is an error of phenylalanine and tyrosine (amino acid metabolism).
633 - 2. T.
633 - 3. F. It is detected by the ferric chloride test and confirmed by paper chromatography.
633 - 4. T.
633 - 5. T.

634 - 1. b.
634 - 2. a, b.
634 - 3. a.
634 - 4. c.
634 - 5. a.
634 - 6. b.
634 - 7. b.

635 - 1. F. Neither test detects β-hydroxybutyric acid.
635 - 2. T.
635 - 3. T.
635 - 4. F. It is read as negative, small, moderate, and large amounts.
635 - 5. T.
635 - 6. F. False positive reactions occur.
635 - 7. Only acetoacetic acid.
635 - 8. Both acetone and acetoacetic acid.

636 - 1. The test strips for hemoglobin are impregnated with orthotolidine which turns blue when oxidized by peroxide in the presence of hemoglobin.
636 - 2. Citrate buffers in the test strips.
636 - 3. Hemoglobin and hyoglobin.
636 - 4. Ascorbic acid in urine will inhibit the reactions.

637 - 1. Phenylketonuria is an error of protein metabolism which results in the accumulation of phenylalanine in the blood and consequent mental retardation.
637 - 2. Reagent sticks for detecting phenylketones, principally phenylpyruvic acid.
637 - 3. Ferric ammonium sulfate, magnesium sulfate, and syclohexylsulfamic acid.
637 - 4. Urine containing salicylates and phenothiazine derivatives will cause the strips to turn a pink to purple color and phenylpyruvic acid will give a bluish gray to gray-green color reaction.
637 - 5. Infants fail to show a positive urine test until several weeks after birth. Unless detected early, the infants suffer irreversible brain damage.

638 - 1. F. A culture of *Bacillus subtilis*.
638 - 2. T.
638 - 3. F. Elevated phenylalanine plasma levels may be found before urine levels are increased.
638 - 4. T.
638 - 5. T.
638 - 6. T.
638 - 7. F. Quantitative flourometric phenylalanine determination is the only presently quantitative analysis useful for following dietary treatment.
638 - 8. T.

639 - 1. e.
639 - 2. a.
639 - 3. a.
639 - 4. j.
639 - 5. a.
639 - 6. c.
639 - 7. d.
639 - 8. f.
639 - 9. h.
639 - 10. g.
639 - 11. i.

640 - 1. T.
640 - 2. F. RECs.
640 - 3. F. Most slide tests are based on latex particles agglutination inhibition.
640 - 4. T.
640 - 5. T.
640 - 6. F. The 2-hour test is more sensitive than the 2-minute test.
640 - 7. T.
640 - 8. F. These conditions will invalidate the test results.
640 - 9. T.
640 - 10. T.

641 - 1. Coated red blood cells or latex particles; HCG antiserum.
641 - 2. The HCG present in the urine neutralizes the antiserum so that it fails to agglutinate the HCG coated cells or latex particles.
641 - 3. The red blood cells used as an indicator will settle out to the bottom of the tube in the form of a "doughnut" as shown in the positive examples in figure 5-3.
641 - 4. Quality control.
641 - 5. A useful guide to prognosis of threatened abortion during the first trimester, diagnosis of hydatidiform mole, or choriocarcinoma in moles—malignant testicular tumor.
641 - 6. The beta subunit radioimmunoassay for HCG.

642 - 1. Maintaining an up-to-date book of standard operating *approved* procedures.

642 - 2. Early morning, midstream and examined within 30 minutes.

642 - 3. Mix the urine well.

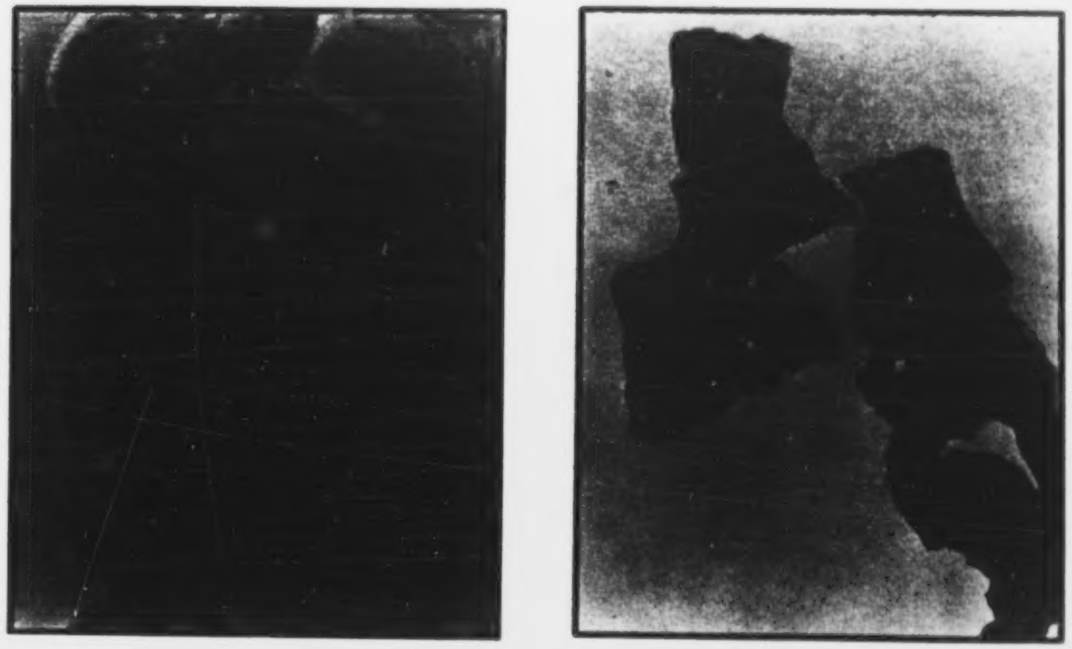
642 - 4. Assures that reagents, reagent strips, and instruments are in working order and also monitors the technique of the individual performing the procedures.

642 - 5. Ames: Tek-Check; Harleco's: Unitrol; Hyland's: Q-Pak Chemistry Urine Control Supplement, Lederle's controls.

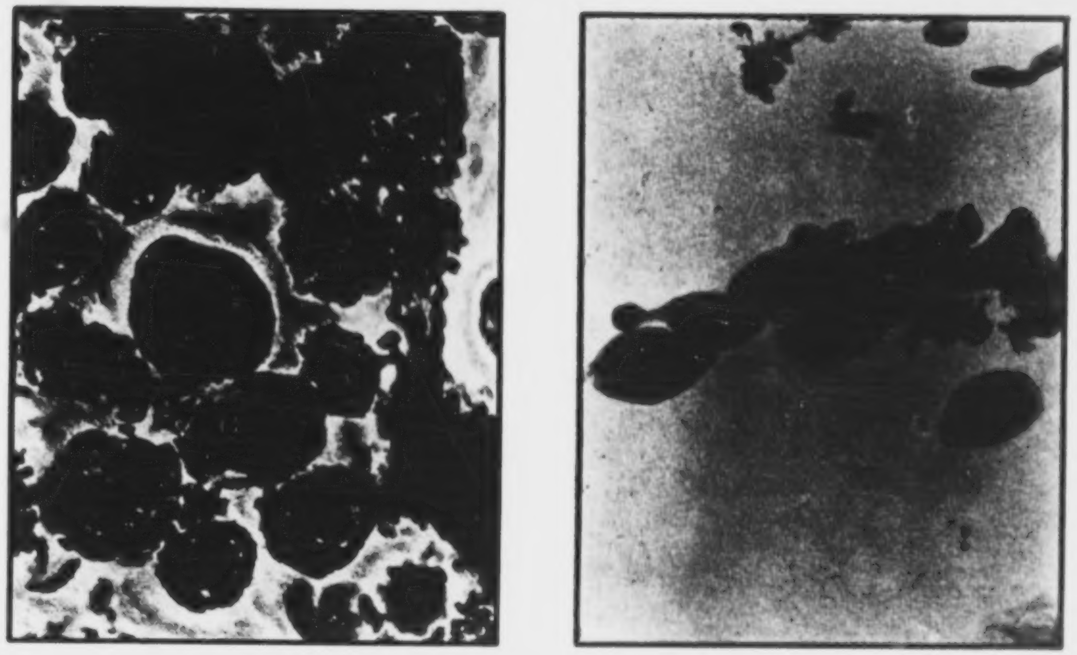
642 - 6. They should be tested in the same manner as the sample.

642 - 7. Internal (daily checks) and external proficiency survey testing.

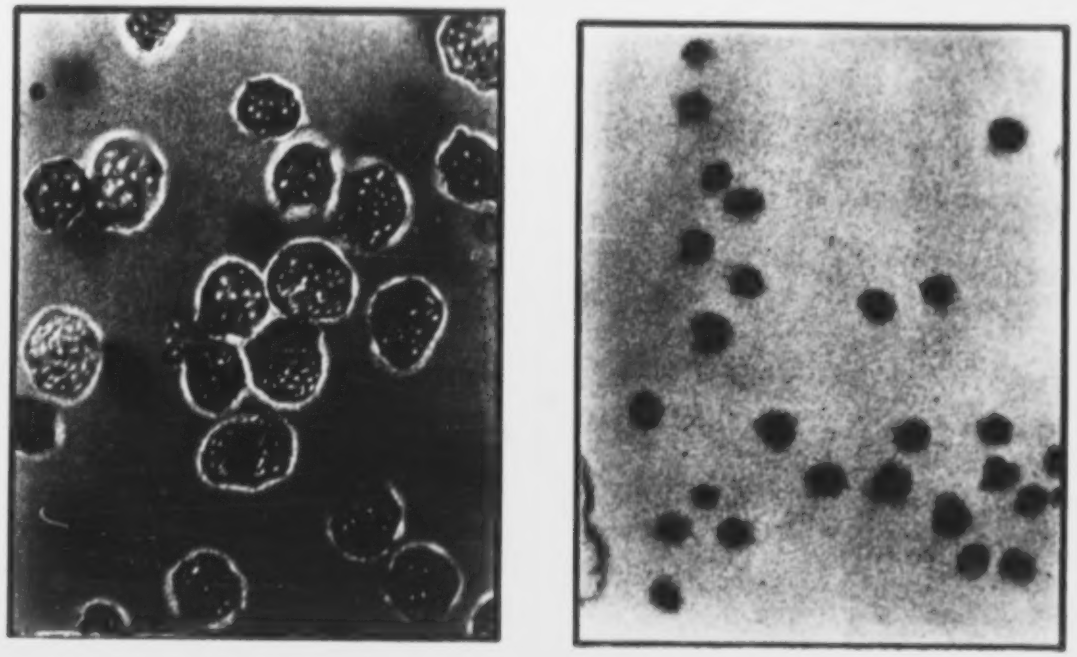
A



EPHITHELIAL CELLS



B



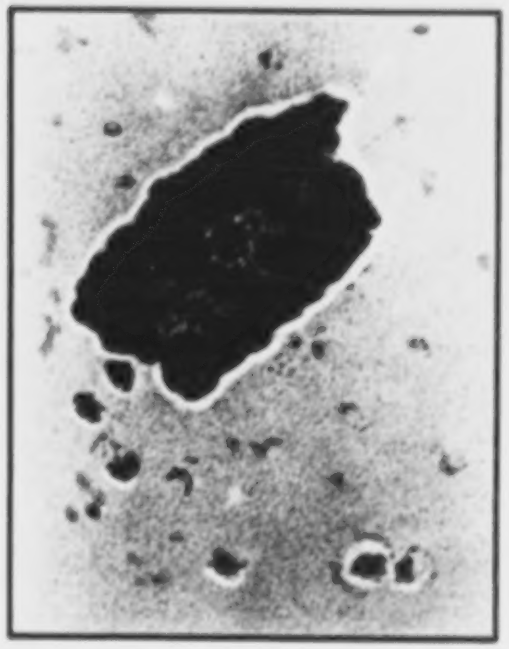
GLITTER

DARKER STAIN

OVAL FAT BODIES

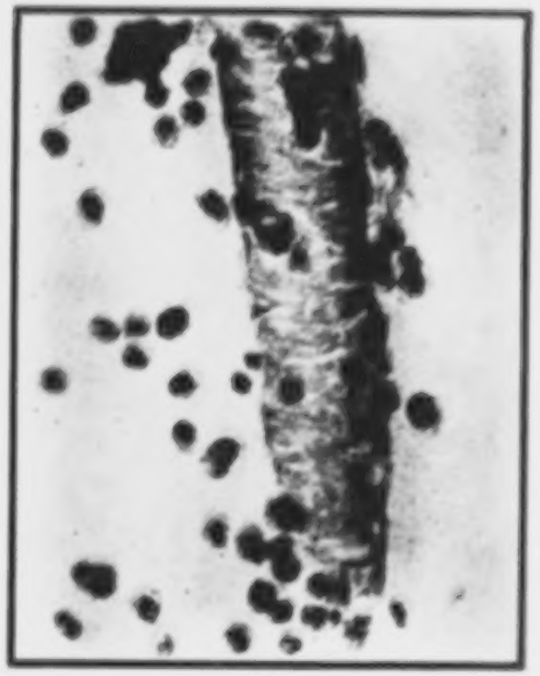
Foldout 1. Casts in urine.

C



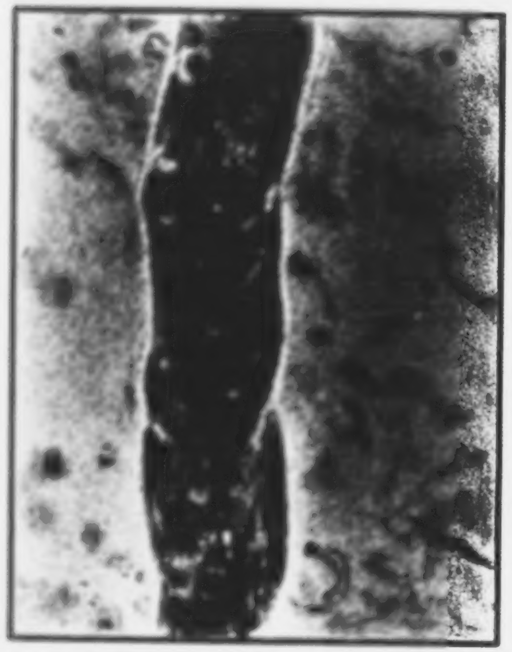
TUBULAR EPHITHELIAL CAST

D

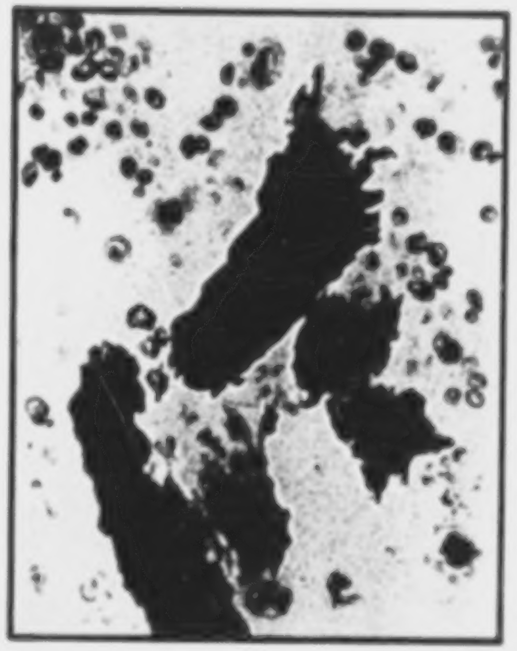


HYALINE CAST

F

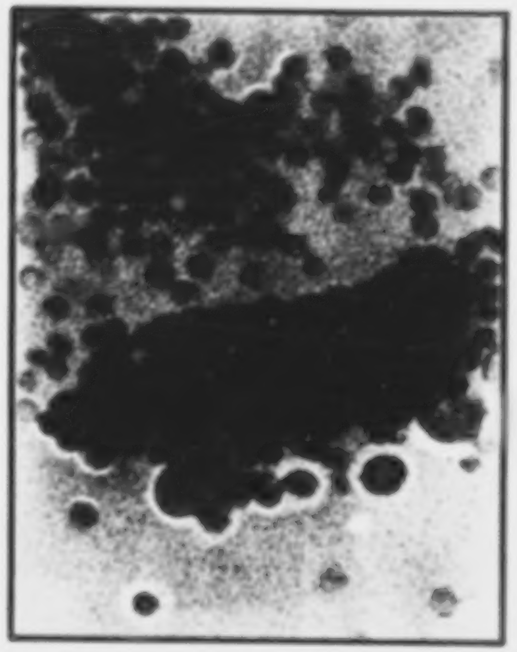


FINE GRANULAR

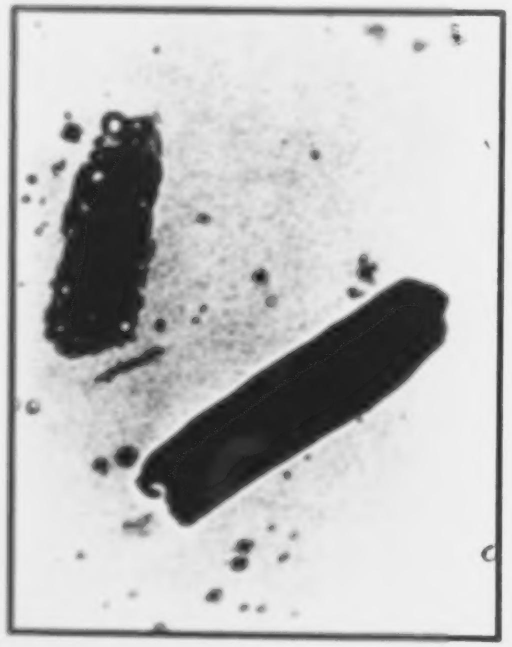


BLOOD CAST

E



RBC CAST



COARSE GRANULAR (UPPER LEFT)
WAXY CAST (LOWER RIGHT)

Foldout 1. Casts in urine (contd).

STOP -

1. MATCH ANSWER SHEET TO THIS EXERCISE NUMBER.

2. USE NUMBER 2 PENCIL ONLY.

90411 04 23

EXTENSION COURSE INSTITUTE
VOLUME REVIEW EXERCISE

LABORATORY PROCEDURES IN URINALYSIS

Carefully read the following:

DO'S:

1. Check the "course," "volume," and "form" numbers from the answer sheet address tab against the "VRE answer sheet identification number" in the righthand column of the shipping list. If numbers do not match, take action to return the answer sheet and the shipping list to ECI immediately with a note of explanation.
2. Note that item numbers on answer sheet are sequential in each column.
3. Use a medium sharp #2 black lead pencil for marking answer sheet.
4. Write the correct answer in the margin at the left of the item. (When you review for the course examination, you can cover your answers with a strip of paper and then check your review answers against your original choices.) After you are sure of your answers, transfer them to the answer sheet. If you *have* to change an answer on the answer sheet, be sure that the erasure is complete. Use a clean eraser. But try to avoid any erasure on the answer sheet if a all possible.
5. Take action to return entire answer sheet to ECI.
6. Keep Volume Review Exercise booklet for review and reference.
7. If *mandatorily* enrolled student, process questions or comments through your unit trainer or OJT supervisor.
   If *voluntarily* enrolled student, send questions or comments to ECI on ECI Form 17.

DON'TS:

1. Don't use answer sheets other than one furnished specifically for each review exercise.
2. Don't mark on the answer sheet except to fill in marking blocks. Double marks or excessive markings which overflow marking blocks will register as errors.
3. Don't fold, spindle, staple, tape, or mutilate the answer sheet.
4. Don't use ink or any marking other than a #2 black lead pencil.

NOTE: NUMBERED LEARNING OBJECTIVE REFERENCES ARE USED ON THE VOLUME REVIEW EXERCISE. In parenthesis after each item number on the VRE is the *Learning Objective Number* where the answer to that item can be located. When answering the items on the VRE, refer to the *Learning Objectives* indicated by these *Numbers.* The VRE results will be sent to you on a postcard which will list the *actual* VRE *items you missed.* Go to the VRE booklet and locate the *Learning Objective Numbers* for the items missed. Go to the text and carefully review the areas covered by these references. Review the entire VRE again before you take the closed-book Course Examination.

Multiple Choice

1. (600) Prior to establishing Kohler illumination with the binocular microscope, you should

    a. align the condenser.
    b. adjust the diaphragm.
    c. focus the lamp filaments.
    d. adjust for refractive differences of your two eyes.

2. (601) After the illuminating lamp and condenser have been focused, the only adjustment to be made is for the

    a. condenser iris diaphragm.
    b. auxiliary lenses.
    c. color correction filters.
    d. lamp filament focus.

3. (602) To produce the desired reduction in illumination of the back lens, you should adjust the

    a. 10X objective.
    b. condenser iris diaphragm.
    c. neutral density filters.
    d. color correction filter.

4. (602) The intensity of microscopic illumination should be controlled

    a. with adjustment of the lamp iris.
    b. by closing the condenser iris.
    c. with neutral density filters.
    d. by opening the condenser iris.

5. (603) Hyaline casts dissolve most readily in urine of

    a. low specific gravity and high pH.
    b. low specific gravity and low pH.
    c. high specific gravity and high pH.
    d. high specific gravity and low pH.

6. (603) A potential source of error in the use of antibacterial cleansing agents for urine specimen collecting is

    a. bacterial contamination.
    b. increased acidity of the urine specimen.
    c. increased alkalinity of the urine specimen.
    d. rendering the urine specimen sterile before culture.

7. (604) A disadvantage of using formalin as a urine specimen preservative is that it

    a. volatilizes.
    b. extracts steroids.
    c. contains carbon atoms.
    d. inhibits the test for indican.

8. (604) When analyses for nitrogen, amino acids, and delta-amino-levulenic acid are to be made, preserve the urine

    a. with sodium flouride.
    b. with concentrated HCl, pH 3.
    c. by refrigeration preferably.
    d. with thymol.

9. (605) The medullary rays of the kidney are located principally within the

    a. medulla.
    b. capsule.
    c. hylum.
    d. cortex.

10. (605) All of the following are considered essential parts of a nephron unit except the

a. glomerulus.
b. loop of Henle.
c. collecting tubule.
d. proximal convoluted tubule.

11. (605) Each glomerulus consists of Bowman's capsule and

a. ducts of Bellini.
b. Malpighian tuft.
c. afferent arteriole.
d. efferent arteriole.

12. (606) In the absence of compensating factors, what effect does a rise in blood pressure have on glomerular filtration rate?

a. Increases.
b. Decreases.
c. No effect.
d. Variable.

13. (606) Most of the water in glomerular filtrate is absorbed in the

a. glomerulus.
b. loop of Henle.
c. distal convoluted tubules.
d. proximal convoluted tubules.

14. (606) The kidneys are able to maintain electrolyte balance in the body by all the following ways except one. Which choice is the exception?

a. Ammonium ions replace sodium ions.
b. Hydrogen ions are excreted by the tubules.
c. Bicarbonate ions are excreted from the tubules.
d. Ketone bodies are oxidized to corresponding organic acids.

15. (607) Which term indicates the pressure of abnormal amounts of urinary constituents in the blood?

a. Uriticaria.
b. Acidosis.
c. Uroerythria.
d. Uremia.

16. (607) Renal amyloidosis is an aspect of which tubular disorder?

a. Mercury poisoning.
b. Tuberculosis.
c. Gout.
d. Multiple myeloma.

17. (608) The maximum daily output of urine for adults should not normally exceed

a. 1,000 ml.
b. 1,500 ml.
c. 2,000 ml.
d. 3,000 ml.

18. (608) A term associated with a decreased urinary output is

a. oliguria.
b. anuria.
c. polyuria.
d. hematuria.

19. (608) The most valuable urine specimen to a physician is usually

a. a late-evening specimen.
b. a mid-afternoon specimen.
c. a first-morning specimen.
d. one taken at random.

20. (609) When the urine is red, you

a. may assume that the blood is normal.
b. may assume the presence of pathology.
c. should be alert to the possibility of pathology.
d. should attribute the variation to the patient's diet.

21. (609) Which of the following is *least* likely a cause of color pigments in normal urine?

a. Bile pigments.
b. Urates and phosphates.
c. Drugs or their metabolites.
d. Vegetable pigments from diets.

22. (610) An aged urine specimen becomes alkaline because of the formation of

a. ammonia.
b. *Pseudomonas*.
c. urea.
d. *Proteus*.

23. (610) What type of reaction (pH) would most likely be indicated in conditions of gout, chronic nephritis, tuberculosis, fever, and leukemia?

a. Strongly alkaline.
b. Strongly acid.
c. Neutral.
d. Weakly acid.

24. (611) With which two indicators are the pH portion of reagent strips impregnated?

a. Methyl orange and thymol blue.
b. Methyl red and bromphenol blue.
c. Phenol red and bromthymol blue.
d. Methyl red and bromthymol blue.

25. (611) Which of the following is least likely to be a source of error in using the pH paper strip?

a. Immediate draining by touching side of container.
b. Leaching reagent by soaking.
c. Acid or alkaline fumes.
d. Previously wet strips.

26. (612) In using a urinometer to measure specific gravity, the correction factor for each 3° C higher or lower than calibration temperature is

a. ±1.001.
b. ±0.100.
c. ±0.010.
d. ±0.001.

27. (613) A direct relationship exists between the refractive index of a solution and the concentration of

a. specific solutes.
b. dissolved solids.
c. specific gravity.
d. colloidal suspensions.

28. (613) Refractive index is the ratio of the velocity of light in air to the velocity of light in a

a. solution.
b. concentrated solution.
c. dilute solution.
d. saturated solution.

29. (614) Which of the following measurements reflects the ability of the kidney to conserve or excrete water?

a. Urine osmolality.
b. Free water clearance.
c. Glomerular filtration rate.
d. Ratio of plasma to urine osmolality.

30. (614) The normal range of urine osmolal concentration for a patient on a normal fluid and food intake is from

a. 200 to 400 mOsm/kg water.
b. 400 to 500 mOsm/kg water.
c. 500 to 850 mOsm/kg water.
d. 800 to 1100 mOsm/kg water.

31. (614) In the measurement of osmotic pressure, a one molal solution 1000 mOsm/kg, depresses the freezing point how many degrees centrigrade below the 0° C freezing point of water?

a. 1.76° C.
b. 1.86° C.
c. 1.90° C.
d. 1.96° C.

32. (615) An object in urine which appears to be budding is probably a

a. yeast cell.
b. spermatozoon.
c. white blood cell.
d. dividing erythrocyte.

33. (616) The addition of dilute acetic acid will clear the urine of

a. uric acid.
b. amorphous urates.
c. amorphous carbonates.
d. leukocytes.

34. (616) What are the two main stains that comprise the Sternheimer-Malbin stain?

a. Crystal violet and eosin.
b. Methyl blue and safranine.
c. Cresyl violet and safranine.
d. Crystal violet and safranine.

35. (617) The nuclear structure of the leukocytes in the urinary sediment may be obscured by the presence of

a. phosphates.
b. albumin.
c. urates.
d. hyalin.

36. (617) Which choice is correct concerning glitter cells in urinary sediment?

a. They contain many bacteria.
b. They have no diagnostic value.
c. They stain orange with Sudan III.
d. They are characteristic of degenerative tubular disease.

37. (618) Which listed characteristic of a urine specimen least affects the appearance of RBCs?

a. pH of specimen.
b. Specific gravity.
c. Age.
d. Color.

38. (618) In a hypertonic medium, red blood cells will

a. hemolyze.
b. crenate.
c. remain intact.
d. produce ghost forms.

39. (618) In a dilute hypotonic urine, what forms of erythrocytes will most likely be observed?

a. Hemolyzed.
b. Intact.
c. Ghost forms.
d. Crenated.

40. (619) Another term for transudation in describing a cast is

a. hyaline.
b. epithelial.
c. waxy.
d. transitional.

41. (619) With the Sternheimer-Malbin stain, hyaline material stains

a. red.
b. green.
c. pink.
d. blue.

42. (619) With the Sternheimer-Malbin stain, red cells in a red cell inclusion cast appear

a. orange.
b. dark blue.
c. pale pink.
d. pale lavender.

43. (619) Which of the following casts is most likely to be found in urine of high specific gravity following a period of oliguria?

a. Coarse granular.
b. Fine granular.
c. Waxy.
d. Fresh tubular epithelial.

44. (619) Which choice is correct concerning broad casts in urine?

a. Result from urinary stasis.
b. Indicate a swollen tubular epithelium.
c. Occur primarily in urine from children.
d. Result from increased function of nephron units.

45. (620) "Highly refractile prisms varying in size and resembling coffin lids" describes which of the following crystals?

a. Uric acid.
b. Ammonium oxalate.
c. Ammonium biurate.
d. Triple phosphate.

46. (620) Uric acid crystals are soluble in

a. trichloroacetic acid.
b. acetic acid.
c. hydrochloric acid.
d. sodium hydroxide.

47. (620) Amorphous urates may be differentiated from amorphous phosphates by which of the following criteria?

a. Amorphous urates are soluble in alkali.
b. Amorphous phosphates are soluble in alkali.
c. Amorphous urates are soluble in acetic acid.
d. Amorphous phosphates dissolve more readily upon warming.

48. (621) Select the correct choice concerning leucine and tyrosine crystals.

a. Commonly found in the urine.
b. Both soluble in boiling acetic acid.
c. Both soluble in dilute hydrochloric acid.
d. Formed as the result of serious liver damage.

49. (621) The Morner reagent is used to test for

a. tyrosine.
b. cystine.
c. leucine.
d. sulfa derivatives.

50. (621) A solution will give a red color with naphthoquinone-4-sodium sulfonate sulfite reagent (Sullivan test) if it contains

a. leucine.
b. isoleucine.
c. cystine.
d. cholesterol.

51. (621) Which crystals have a "missing corner" and appear in an acid specimen as large, flat, colorless plates?

a. Leucine.
b. Tyrosine.
c. Cholesterol.
d. Sulfonamides.

52. (622) Nonserum proteins from the ureters, bladder, urethra, and prostate with an electrophoretic mobility slightly greater than that of albumin are called

a. proteoses.
b. plasma proteins.
c. Bence-Jones proteins.
d. "Tamm-Horsfall" mucoproteins.

53. (622) All of the following are causes of proteinuria and are not related directly to kidney diease except

a. renal trauma.
b. febrile conditions.
c. urogenital tract bleeding.
d. extraordinary physical exercise.

54. (623) Taking into account normal fluctuations in urine volume, the upper limit of "normal" for urinary protein in mg/100 ml of urine is

a. 1 to 15.
b. 7 to 10.
c. 7 to 70.
d. 70 to 100.

55. (623) Which listed factor has the least influence on the normal range of protein in a 24-hour urine specimen?

a. Weight.
b. 24-hour urine volume.
c. Method of determination.
d. Diet.

56. (624) Massive doses of penicillin will cause false positive reactions with all of the following tests except

a. sulfosalicylic acid.
b. nitric acid.
c. heat and acetic acid.
d. reagent strip.

57. (624) Which of the following tests gives a false positive reation with highly buffered alkaline urine?

a. Trichlorocetic acid.
b. Sulfosalicylic acid.
c. Heat and acetic acid.
d. Reagent strip.

58. (625) The optimal pH for protein precipitation is between

a. 2 and 3.
b. 4 and 5.
c. 6 and 7.
d. 8 and 9.

59. (625) In the heat and acetic acid screening test for urine protein, the acetic acid

a. dissolves urates.
b. reacts with carbonates.
c. prevents negative reactions.
d. forms an acid reagent buffered at pH 4.

60. (626) The reagent strip for protein is impregnated with which of the following indicators?

a. Thymol blue.
b. Bromphenol blue.
c. Tetrabromphenol blue.
d. Tetrathymol blue.

61. (626) In view of possible false positive reactions for protein with strongly alkaline urine, you should consider the

a. urine color.
b. specific gravity.
c. urine sugar.
d. urine pH.

62. (627) All of the following reducing sugars are pentoses except

a. lactose.
b. arabinose.
c. xylose.
d. rhamnose.

63. (627-628) Which of the following will not reduce Benedict's reagent?

a. Xylose.
b. Sucrose.
c. Lactose.
d. Glucose.

64. (628) A "pass through" reaction with the urine sugar test tablet indicates that reducing substances are

a. negative.
b. 1 percent or less.
c. 2 percent.
d. more than 2 percent.

65. (628) Ascorbic acid, which interferes with urine glucose tests, may result from

a. iron therapy.
b. a diseased pancreas.
c. endocrine disorders.
d. abnormal amounts of protein.

66. (629) Lactose may be identified by all the following procedures except

a. mucic acid test.
b. Tauber's test.
c. osazone test.
d. paper chromatography.

67. (629) Which of the following nonglucose reducing substances if not eliminated from the diet will cause rapid physical and mental deterioration in infants?

a. Xylose.
b. Fructose.
c. Galactose.
d. Glucose.

68. (629) The Benedict's test or Clinitest for reducing substances would be of great advantage when screening children for

a. metabolic disorders.
b. glucosuria.
c. kidney disease.
d. diabetes mellitus.

69. (630) How long does it take to establish a chemical equilibrium between the alpha and beta forms of d-glucose in water?

a. 1 hour.
b. 30 minutes.
c. 15 minutes.
d. 5 minutes.

70. (630) In the glucose oxidase reaction, hydrogen peroxide produced in the reaction with glucose oxidizes which of the following reagents to produce a blue color?

a. Benzidine.
b. Gluconic acid.
c. Orthotolidine.
d. Orthotoluidine.

71. (630) What substance below causes a false negative glucose oxidase reaction and false positive Benedict's reaction?

a. Lactose.
b. Coca Cola.
c. Vitamin C.
d. Antibiotics.

72. (630) Which of the following conditions can be missed if glucose oxidase is used as the only screening test?

a. Pentosuria.
b. Glucosuria.
c. Fructosuria.
d. Lactosuria.

73. (631) The urine sample must be fresh when making the urobilinogen test because if urine stands too long, urobilinogen is converted to

a. urobilin.
b. bilirubin.
c. stercobilinogen.
d. mesobilinogen.

74. (631) In the Ehrlich reaction, urobilinogen reacts with which of the following reagents in HCl to form a red color?

a. Acetoacetic acid.
b. Para-benzaldehyde.
c. Beta-hydroxybutyric acid.
d. Para-dimethyl-amino benzaldehyde.

75. (631) In the Ehrlich's test for urobilinogen, which of the following may be added to remove bile pigments?

a. Ascorbic acid.
b. Barium hydroxide.
c. Barium chloride.
d. Sodium acetate.

76. (631) Urobilinogen may be distinguished from porphobilinogen on the basis of solubility in

a. calcium chloride.
b. sodium acetate.
c. water.
d. chloroform.

77. (632) Which of the following is not one of the porphyrin group of compounds?

a. Porphobilinogen.
b. Urobilinogen.
c. Coproporphyrin.
d. 5-aminolevulinic acid.

78. (632) Coproporphyrin III excretion is markedly increased in which of the following conditions?

a. Lead poisoning.
b. Porphyrin metabolism.
c. Arsenic poisoning.
d. Bilirubinemia.

79. (633) Which of the following is a black pigment excreted in some cancer states?

a. Gentistic acid.
b. Indican.
c. Melanin.
d. Phenol.

80. (633) Homogentistic acid is usually detected by which of the following tests?

a. Ferric chloride.
b. Hemosiderin.
c. Abermayer.
d. Salicylate.

81. (634) Which of the following reagents in the reagent tablet for bilirubin serve to produce effervescence and enhance solubility?

a. Sulfosalicylic acid and sodium hydroxide.
b. Sulfosalicylic acid and sodium bicarbonate.
c. Sulfosalicylic acid and p-toluene sulfonate.
d. Stabilized p-nitrobenzene p-toluene sulfonate and sodium bicarbonate.

82. (634) False positive reactions may occur using the reagent strips for bilirubin with urine from patients taking large doses of

a. penicillin.
b. pyridium.
c. iron.
d. chlorpromazine.

83. (635) The reagent used in the Acetest® (or modified Rothera test) is

a. sodium azide.
b. sodium nitroprusside.
c. para-benzaldehyde.
d. diazo reagent.

84. (636) In the test for occult blood using the test strip, a blue color results from hemoglobin catalyzing the oxidation of

a. benzidine.
b. orthotoluidine.
c. hemosiderin.
d. orthotolodine.

85. (636) Using the test strips, which of the following substances may produce a false negative reaction for occult blood?

a. Ascorbic acid.
b. Pyridium.
c. Diacetic acid.
d. Acetoacetic acid.

86. (637) What is the principal phenylketone detected by the reagent strips?

a. Phenothiazine.
b. Phenylpyruvic acid.
c. Phenylpyruvic glutamic acid.
d. Phenylpyruvic oxalacetic acid.

87. (637) Reagent strips are inadequate for PKU testing of infants 24 hours after birth because

a. the strips lack specificity.
b. the strips lack sensitivity.
c. affected infants fail to show a positive urine test until several weeks after birth.
d. affected infants fail to show a positive urine test until several months after birth.

88. (115) The Guthrie test is based upon the principle that the growth of which of the following organisms is inhibited by beta-2-thienylalanine?

a. *Bacillus subtilis.*
b. *Bacillus anthracis.*
c. *Bacillis stearothermophilus.*
d. *Bacillus cereus.*

89. (638) Which of the following may be used to evaluate the progress of a patient receiving dietary treatment for PKU disorder?

a. The Guthrie test.
b. The fluorometric method.
c. Thin layer chromatography.
d. Ferric chloride or reagent strips.

90. (639) The alpha units of the HCG compound are considered nonspecific and are shared by all of the following *except*

a. luteinizing hormone (LH).
b. human placental lactogen (HPL).
c. thyroid stimulating hormone (TSH).
d. follicle stimulating hormone (FSH).

91. (639) A bioassay test for gonadotropin which uses female mice is the

a. Aschheim-Zondek test.
b. Friedman test.
c. Xenopus laevis test.
d. Rana pipiens test.

92. (640) Which of the following terms best describes the slide test for pregnancy involving latex particles coated with human chorionic gonadotropin?

a. Agglutination.
b. Agglutination-inhibition.
c. Precipitation.
d. Autolytic.

93. (640) How long is HCG in urine considered stable at room temperature?

a. 72 hours.
b. 24 hours.
c. 12 hours.
d. 6 hours.

94. (640) Authorities expect erroneous results in what percent of the slide tests for pregnancy?

a. 1 to 2 percent.
b. 3 to 4 percent.
c. 5 to 6 percent.
d. 7 to 8 percent.

95. (640) Urine specimens for pregnancy testing that must be preserved are best preserved by

a. refrigeration.
b. boric acid.
c. freezing.
d. formalin.

96. (641) In the 2-hour pregnancy test, urine specimens containing HCG neutralize the HCG antiserum so that it

a. neutralizes the HCG coated cells.
b. fails to agglutinate the HCG coated cells.
c. reduces the agglutination of HCG coated cells.
d. increases the agglutination of the HCG coated cells.

97. (641) All of the following may be considered reasons for a quantitive HCG assay except

a. ectopic pregnancy.
b. choriocarcinoma in males.
c. diagnosis of hydatidiform mole.
d. guide to prognosis of threatened abortion during the first trimester.

98. (641) A test reserved for study of patients being treated for choriocarcinoma or hydatidiform mole is

a. quantitative HCG.
b. beta subunit radioimmunoassay for HCG.
c. alpha subunit radioimmunoassay for HCG.
d. beta subunit radioimmunoassay for FSH.

99. (642) Primary consideration in initiating quality control in urinalysis is

a. tailoring the section to your own techniques.
b. discarding all reagents that are 10 days old.
c. maintenance of standard operating approved procedures.
d. maintenance of standard deviation on urinalysis workload.

100. (642) The use of reference samples in urinalysis quality control provides all the following controls except one. Which is the exception?

a. Assures the working order of reagents.
b. Assures the working order of instruments (equipment).
c. Monitors the techniques of the manufacturers of each test.
d. Monitors the technique of the individual performing the procedures.

STUDENT REQUEST FOR ASSISTANCE

PRIVACY ACT STATEMENT

AUTHORITY: 44 USC 3101. PRINCIPAL PURPOSE(S): To provide student assistance as requested by individual students. ROUTINE USES: This form is shipped with every ECI course package. It is utilized by the student, as needed, to place an inquiry with ECI. DISCLOSURE: Voluntary. The information requested on this form is needed for expeditious handling of the student's need. Failure to provide all information would result in slower action or inability to provide assistance.

SECTION I: CORRECTED OR LATEST ENROLLMENT DATA: MAIL TO ECI GUNTER AFS ALA 36118

1. THIS REQUEST CONCERNS COURSE ☐
2. TODAY'S DATE ☐
3. ENROLLMENT DATE ☐
4. PREVIOUS SERIAL NUMBER ☐
5. SOCIAL SECURITY NUMBER ☐☐☐–☐☐–☐☐☐☐
6. GRADE/RANK ☐
7. INITIALS ☐ ☐ LAST NAME ☐
8. OTHER ECI COURSES NOW ENROLLED IN
9. ADDRESS: (OJT ENROLLEES - ADDRESS OF UNIT TRAINING OFFICE/ALL OTHERS - CURRENT MAILING ADDRESS) ZIP CODE ☐☐☐☐☐
10. NAME OF BASE OR INSTALLATION IF NOT SHOWN ABOVE:
11. AUTOVON NUMBER ☐
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SECTION II: Old or INCORRECT ENROLLMENT DATA

1. NAME: 2. GRADE/RANK: 3. SSAN:
4. ADDRESS: 5. TEST OFFICE ZIP/SHRED:

SECTION III: REQUEST FOR MATERIALS, RECORDS, OR SERVICE

(Place an "X" through number in box to left of service requested)

ADDITIONAL FORMS 17 available from trainers, OJT and Education Offices, and ECI. The latest course workbooks have a Form 17 printed on the last page.

1	EXTEND COURSE COMPLETION DATE. (Justify in Remarks)
2	SEND VRE ANSWER SHEETS FOR VOL(s): 1 2 3 4 5 6 7 8 9 - ORIGINALS WERE: NOT RECEIVED, LOST, MISUSED
3	SEND COURSE MATERIALS (Specify in remarks) - ORIGINALS WERE: NOT RECEIVED, LOST, DAMAGED.
4	COURSE EXAM NOT YET RECEIVED. FINAL VRE SUBMITTED FOR GRADING ON (Date):
5	RESULTS FOR VRE VOL(s): 1 2 3 4 5 6 7 8 9 NOT YET RECEIVED. ANSWER SHEET(s) SUBMITTED ON (Date):
6	RESULTS FOR CE NOT YET RECEIVED. ANSWER SHEET SUBMITTED TO ECI ON (Date):
7	PREVIOUS INQUIRY (ECI FORM 17, LTR, MSG) SENT TO ECI ON:
8	GIVE INSTRUCTIONAL ASSISTANCE AS REQUESTED ON REVERSE:
9	OTHER (Explain fully in remarks)

REMARKS: (Continue on Reverse)

OJT STUDENTS must have their OJT Administrator certify this request. ALL OTHER STUDENTS may certify their own requests.	I certify that the information on this form is accurate and that this request cannot be answered at this station. (Signature)

ECI FORM 17 JUN 77 PREVIOUS EDITIONS MAY BE USED

SECTION IV: REQUEST FOR INSTRUCTOR ASSISTANCE

NOTE: Questions or comments relating to the accuracy or currency of textual material should be forwarded directly to preparing agency. Name of agency can be found at the bottom of the inside cover of each text. All other inquiries concerning the course should be forwarded to ECI.

VRE ITEM QUESTIONED: MY QUESTION IS:

Course No. ____________

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Answer You Chose
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Has VRE Answer Sheet
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